Detection of *sul1* resistance gene in *Acinetobacter baumannii* from different Clinical cases

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Abstract:

Acinetobacter baumannii, is widespread, opportunistic pathogen that has conceded as a global threat because of high levels of resistance to many antibiotics. Many study report the molecular characterization of sulphonamide resistance genes and gene cassettes associated with class I integrons in various Enterobacteriaceae including extended spectrum b-lactamase (ESBL) producers. *sul1*, *sul2* and *sul3* encoding dihydropteroate synthases and more than 20 dihydrofolate reductase (dfr) genes have been described. Both groups of genes are associated with class 1 integrons residing in plasmids and/or the bacterial chromosome.

Nineteen isolates of *Acinetobacter baumannii* were obtained from various clinical cases including sputum (8), blood (5), wound swab (4), urine (1) and throat swab (1).

The antimicrobial susceptibility results revealed that *Acinetobacter baumannii* isolates were resistance to Ceftazedim, Ciprofloxacin, Cefotaxim, Cefepime, Ampicillin and Agumantin (100 %), gentamicin (94.7%), and Imipenim (68.4%).

Molecular investigation of (*sul1)* gene exhibited that (*sul1*) gene was detected in 17 (98%) of isolates. all isolates was multidrug resistance. In other hand, *sul1* gene was not detected in strains 5, 13 only and both of them were resistant to 7 antibiotics and sensitive to Imipenem. The gel electrophoresis revealed that the molecular weight of the (*sul1)* gene was (822) bp.

Introduction:-

Acinetobacter baumannii is a cosmopolitan threat for healthcare sitting (Wareth et al. 2021).*Acinetobacter baumannii* is a notorious, non-motile, pleomorphic bacillus and gram-negative member of the gamma proteobacteria (Bahavarnia et al.2020a). It is the main cause of nosocomial infections in bloodstream, urinary tract, wounds and in lungs leading to pneumonia (Bahavarnia et al.2020b), skin and urinary tract infections and secondary meningitis , *Acinetobacter baumannii* is the main pathogen causing severe acute pancreatitis (SAP) secondary infection (Tian et al. 2020).And high mortalities in intensive care units (ICUs) (Kurihara et al. 2020).

The emergence and prevalence of *Acinetobacter baumannii* have become major health challenge worldwide; therefor the World Health Organization (WHO) identified the rapid spread of *Acinetobacter baumannii,* and *Pseudomonas aeruginosa*, as a global crisis (Theriault et al. 2021) (WHO, 2020). The majority of infections in the United States were caused by *A. baumannii* and *P. aeruginosa* (Cai et al. 2017) Urinary tract infections (UTIs) are the most common infections in the United States caused by Gram-negative bacteria such as *Acinetobacter baumannii* (Shields et al. 2021). A study found that the most frequent antibiotic -resistant pathogen was *Acinetobacter baumannii* (in 54 patients [46%]), from 152 patients in 95 hospitals in 16 countries in North America, South America, Europe, and Asia (Bassetti et al. 2021).This pathogen possesses a broad range of virulence factors and mechanisms such as: outer membrane vesicles (OMV), biofilm formation and cytotoxicity (Dahdouh et al. 2016), It has the ability to resistant to a wide range of antimicrobial agents (Howard et al. 2012).Clinically, huge outbreaks of *A. baumannii* have been reported worldwide. *A. baumannii* was the most frequently described pathogenic bacteria as cause of health care-associated infections in Italian during 2006-2017 as reported by Italian Nosocomial Infections Surveillance in Intensive Care Units (ICUs) network (SPINUTI) (Zarrilli et al. 2021). Europe and the Mediterranean regions harbored the highest value of MDR *A. baumannii* strains, reported by a global surveillance program (Flamm et al. 2016). Antibiotic resistance is a serious problem. More than 50% of the Vietnamese isolates harbored genes that confer resistance to sulfonamides such as *Sul1* gene in *A. baumannii* strains in Southeast Asia hospitals (Wareth et al. 2021). *Sul1* is gene coding for sulphonamid antibiotic resistance; it is commonly used to treat bacterial infections (Vijayashree et al. 2018). Many

study report the molecular characterization of sulphonamide resistance genes and gene cassettes associated with class I integrons in various Enterobacteriaceae including extended spectrum b-lactamase (ESBL) producers and enteric pathogens such as Salmonella and Shigella. *Sul1 was* the main antibiotic resistance gene (ARG) in water and there was correlation between *Sul1*and concentrations of the antibiotics (Yuan et al. 2019). Pérez-Etayo et al reported that significant correlations between ARBs and ARGs were detected, Multidrug resistance was found in 31.79% of the isolates of coliforms and *E. coli*. The most antibiotic resistance bacteria(ABRs) were 62.64% resistant to CFZ (Cefazolin), 54.97% resistant to AMP (ampicillin), and 45.05% resistant to AMC (amoxicillin/clavulanic acid) (Pérez-Etayo et al. 2020). Aims of study was detection *sul1* gene in *Acinetobacter baumannii* that have multidrug resistance isolated from different clinical cases.

2. Material and Methods

2.1: Collection of samples

Professor dr. Rana Mujahid Abdulla (Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad was kindly provided our project with a total of 19 isolates of *Acinetobactor baumannii* (The clinical samples including sputum (8), blood (5), wound swab (4), urine (1) and throat swab (1)).

To detect the sensitivity test of *Acinetobactor baumannii*. isolates according to Disk diffusion method Kirby-Bauer) according to Hudzicki (2016), adjust the turbidity of bacterial suspension to a 0.5 McFarland standard, then dip a sterile swab into the bacterial suspension and streak the dried surface of Mueller-Hinton agar over the entire agar surface after place the antibiotic dicks on the surface of Mueller-Hinton agar using forceps. Then the plates were incubated at 37ºc for 18-24 hours. Following incubation, measuring of inhibition zone around antibiotic discs by rule.

2.2: Extraction of DNA:

DNA was extracted from bacterial strains by using DNA kit Extraction, according to the manufacturer's instructions of Wizard genomic DNA Purification kit (Wizard Genomic DNA Purification Kit, Promega).

2.3: Concentration of DNA:

To detect the concentration of extracted DNA, Quantus Florometer was used in order to reveal the righteousness of samples for downstream applications. For 199 µl of diluted Quanty Flour Dye, 1 µl of DNA was mixed. DNA concentration values were detected after 5min incubation at room temperature.

2.4: Preparation of primers:

According to the instructions of the manufacturer (Macrogen Company), the stock solution of primers (*Sul1*-F: 5`-TTCGGCATTCTGAATCTCAC-3`, *Sul1* –R: 5`-ATGATCTAACCCTC GGTCTC-3`) 822 bp (Askari et al., 2019) was prepared, by using sterile distilled deionized nuclease free water to obtain a concentration of 100 pmol/µl as a stock solution. By adding 10µl from each stock solution to 90 µl of Distilled Water, the solution of each initiator was present separately at 10 pmol/µl and mixed well with Vortex mixture. Keeping at -20°C, after removing it from ice the initiator solution was mixed well by using the Vortex carburetor prior to use.

2.5: Prepare PCR mixture:

The mixture consisted of (5µl GO Taq Green Master Mix Bioneer (Korea), 5 µl of DNA template, 2 µl of F-Primer, 2 µl of R-Primer, 6 µl of Deionized Sterile Distilled Water. For detection *Sul1* gene, the optimum conditions were: for initiation one cycle for 5 minutes at 95° C, for DNA denaturation 30 cycles for 30 seconds at 95°C, for annealing to DNA 30 seconds at 55°C, 30 seconds at 72°C to elongate and then for final elongation only one cycle for 7 minutes and at 72°C.

2.6: Agarose Gel Electrophoresis:

Agarose Gel Electrophoresis was performed after PCR amplification, to confirm the presence amplification, by loading 10 µl of the PCR products to the wall directly. It was performed at 100 volt for 75 minutes; by using gel imaging system (Gel Imaging System Major Science, Taiwan) the bands in gel were visualized (Sambrook and Rusell, 2001).

Results and Discussion:

A total of 19 clinical isolates of *Acinetobactor baumannii* were chosen from diverse anatomical origin of patients who were suffered from various diseases. The clinical samples including sputum (8), blood (5), wound swab (4), urine (1) and throat swab (1).

All bacterial strains were oxidase negative and catalase positive, examined phenotypically by culturing on MacConkey agar, showed smooth, mucoid and pink colonies. The isolates showed smooth, mucoid colonies, white trend to grey color and hemolytic bacteria in blood agar. After staining by gram stain, the strains of *A. baumannii are* characterized by coccobacillary shape. Vitic 2 system was used for final identification.

Acinetobactor baumannii is becoming a troublesome issue worldwide (Luo et al. 2021). The World Health Organization (WHO) mentioned *Acinetobactor baumannii* strains in the priority pathogens list for which

innovative new antimicrobial agents are extremely required (Neshani et al., 2020). *Acinetobactor baumannii* have been reported in different regions from around the world, in recent years *Acinetobactor baumannii* has been related to significant morbidity and mortality rates (Kyriakidis et al. 2021). Its overall mortality can be as high as 56.2% (Mohd Sazlly Lim et al. 2019). Increased happening of resistant *A. baumannii* isolates has been detected in the East countries of the Arab faction (Iraq, Jordan, Lebanon, Palestinian, and Syria) (Moghnieh, et al. 2018). Antibiotic multi resistance has considers by The World Health Organization (WHO) to be one of the greatest health threats of the century. (Global Commitment).

Our results showed that all *A. baumannii* clinical isolates were multi-drug resistant (MDR). Resistance to antibiotics was widespread among the *A.* baumannii clinical isolates. Antimicrobial susceptibility test that all of *A. baumannii* isolates were resistant to Ceftazedim, Ciprofloxacin, Cefepime, Cefotaxim, Cifepime, Ampicillin and Augmantin 100% and next largest value were resistant to Gentamycine with 94.7%. Imipenem showed the lowest proportion as 68.4%. The result compared to standard in Clinical Laboratory Standards Institute (CLSI, 2019)

Genotyping detection *sul1* gene revealed that most of *A. baumannii* isolates 17 (98%) were harbored *sul1* gene all isolate was multidrug resistance. In other hand, *sul1* gene was not detected in strains 5, 13 and both of them were resistant to 7 antibiotics and sensitive to Imipenem Table (1).

Table 1: *A. baumannii* clinical strains susceptibility pattern.

No. of isolate	Ceftazedim	Imipinem	Gentamycin	Ciprofloxacin	Cefotaxim	Cifepim	Ampicillin	Augmantin	No. of antibiotic resistance	*sul1* resistance gene
1	R	R	R	R	R	R	R	R	8	+
2	R	R	R	R	R	R	R	R	8	+
3	R	R	R	R	R	R	R	R	8	+
4	R	R	R	R	R	R	R	R	8	+
5	R	S	R	R	R	R	R	R	7	-
6	R	R	R	R	R	R	R	R	8	+
7	R	S	R	R	R	R	R	R	7	+

8	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**7**	**+**
9	**R**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**6**	**+**
10	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**8**	**+**
11	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**7**	**+**
12	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**8**	**+**
13	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**7**	**-**
14	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**8**	**+**
15	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**8**	**+**
16	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**8**	**+**
17	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**8**	**+**
18	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**8**	**+**
19	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**8**	**+**
%	**100**	**68.4**	**94.7**	**100**	**100**	**100**	**100**	**100**		

Antibiotic sensitivity test showed that our study in agreement with Askari et al., they reported: Most *A. baumannii* strains represented *sul1* (78.43%) gene. Moreover, more than 50% of *A. baumannii* strains were resistant to gentamycin (74.50%) (Askari et al., 2019). Current finding in agreement with Hung et al. that stated the isolates of *A. baumannii* were resistant to Ceftazedim, Gentamycin, Ciprofloxacin, Imipenem and Cefepime. (Hung et al. 2012). The result was in agreement with Shafigh et al., the resistant of *A. baumannii* to Imipenim was 100% (Shafigh et al., 2018).

In our study , the resistant to Augmantin antibiotic was 100% , our result in agreement with the research was performed by Sepahvand et al. , who showed that resistant to Augmantin was 100% (Sepahvand et al. , 2017).

Ceftazedim and Imipeninm are belonging to Beta-lactam antibiotics, in our study, the results were 100%, 31% respectively. In study was performed by Shafigh et al., the resistant to Ceftazedim and Imipeninm was 100%, 85% respectively (Shafigh et al., 2018). In comparing with Abdullah and Adnan, the antibiotic resistant pattern to Ceftazedim and Imipeninm was 64%, 43% respectively (Abdullah and Adnan, 2020). Our finding in agreement with study was done by Liu, y. and Liu, x. the antibiotic resistant pattern to Cefotoxim and Cefepim was 100% (Liu, y. & Liu, x. 2015). Whereas the study was performed by Abdullah and Adnan, who showed that the most strains of *A. baumannii* resistant to Cefotoxim and Cefepim in 68%, 50% respectively (Abdullah and Adnan, 2020).

All isolates of *A. baumannii* showed resistant to Ampicillin 100%, this results identical to study conduct by Maraki et al. Antibiotic resistant was more than 92% (Maraki et al., 2016). The finding in agreement with Goudarzi et al., who reported the antibiotics resistant of clinical isolates of *A. baumannii* to Ampicillin, was 100% (Goudarzi et al., 2015). In contrast, Abdullah and Adnan found that the antibiotics resistant to Ampicillin was 78.5% (Abdullah and Adnan, 2020).

In terms of Gentamycin which is belong to Aminoglycosides antibiotics group, in our study, the resistant antibiotic pattern of *A. baumannii* to Gentamycin was 99% , this in agreement with a study conducted by Aliakbarzad et al. was the resistant to Gentamycin was 86% (Aliakbarzad et al., 2014). *A. baumannii* produce Aminoglycoside modifying enzymes which are coded by gene these bacteria therefore *A. baumannii* resistant to Aminoglycosides antibiotics (Shafigh et al., 2018). Whereas other research performed by Abdullah and Adnan, the resistant to Gentamycin was 33% (Abdullah and Adnan, 2020).

Our finding, the resistant of *A. baumannii* Ciprofloxacin was 100%, in agreement with a study done by Ali and Almohaidi, the resistant to Ciprofloxacin was 71.87% (Ali and Almohaidi, 2020). Whereas, Abdullah and Adnan, the resistant to Ciprofloxacin was 50% (Abdullah and Adnan, 2020).

Molecular investigation of *Sul1* gene in of *A. baumannii* exhibited that (*Sul1*) gene was detected in which 17 (89.47%) of isolates were harbored this gene. In other hand to *Sul1* gene was not detected in 5 and 13 isolates. The gel electrophoresis revealed that the molecular weight of (*Sul1*) gene was 822 bp. Fig.1, The strains harbored a *Sul1* gene. *A. baumannii* have high genetic flexibility, these genes playing a role in improving the adaptation to the environment (Wareth et al. 2021). Our finding in agreement with Tavakol, et. al.: *Sul1* (63.63%) was the most commonly detected antibiotic resistance gene in 22 *A. baumanni* strains were isolated from 126 animal meat samples (Tavakol, et. al, 2018). The gene *Sul1* (sulfonamide) is responsible for appearance of resistance against antibiotic (Tavakol, et. al, 2018). Vázquez-López et al. 2020 reported that *A. baumannii* is resistant to sulfonamides in approximately 71.3% of isolates (Vázquez-López et al. 2020). Most *A. baumannii* strains isolated from sheep meat samples represented *Sul1* (78.43%) gene, samples were randomly collected from Isfahan and Shahrekord cities in Iran (Askari & Tajbakhsh, 2019). Mak et

al. mentioned that *Sul1*gene was identified in 10 of the 32 isolates of *A. baumannii* from both outbreak and sporadic cases (Mak et al. 2009).

Liu et al. demonstrated that the adaptation of pathogen *A. baumannii* to a variety of environments due to its high genome variations (Liu et al. 2014). *A. baumannii* is a genomically variable bacterium that has the ability to cause a range of diseases (Bian et al. 2021). Salloum et al. shed light on the relationship between population mobility and the drug-resistant pathogens in developing countries such as Syria. (Salloum et al.2018). Ghaffoori et al. reported the existence of *Sul1*gene accounted for 43 % from 200 samples (Ghaffoori et al, 2020). For multidrug resistance (MDR), our finding showed that most isolates were resistant to 8 antibiotics, 5 isolates were sensitive to Imipenem and one isolate sensitive to Imipenem and Gentamycin. In a study of Adewoyin, et al., who showed that sulphonamides *sul1* (37.1%) was common antibiotics resistance gene, *A. baumannii* isolates were resistant to ceftazidime (12%), cefotaxime (18.8%), cefepime (8.8%), imipenem (2.7%), gentamicin (8.8%) and ciprofloxacin (11%) (Adewoyin, et al., 2021).

Sulphonamides and trimethoprim are inexpensive antibiotics that have a synergistic effect, they have been used in combination (co-trimoxazole) since 1968 for a wide range of clinical including urinary tract infections, enteric bacterial diseases and respiratory tract infections. Plasmid-mediated resistance to sulphonamides and trimethoprim is normally due to the acquisition of novel target enzymes that are naturally resistant: dihydropteroate synthases for sulphonamides and dihydrofolate reductases for trimethoprim. Three resistance genes, *sul1*, *sul2* and *sul3* encoding dihydropteroate synthases and more than 20 dihydrofolate reductase (dfr) genes have been described. Both groups of genes are associated with class 1 integrons residing in plasmids and/or the bacterial chromosome. (Frank et al., 2007). Sulfonamides are used to treat medical cases of animals, such as bacterial pneumonia, bacterial scours, foot rot, calf diphtheria and acute mastitis. They are used to treat beef cattle, non-lactating dairy cattle, swine, chickens, carp, ewes, dogs, quail, horses and turkeys (Faries.& Fajt 2008)..

In the past few decades, veterinary antibiotics have been widely used in many countries to treat disease. This release together with antibiotic-resistant bacteria (ARB) is a great concern recently , primarily because the land application of antibiotic-polluted manure in agricultural practice not only introduced bacteria carrying antibiotic resistance genes (ARGs) into the soil but also had a significant effect on the ARB. The horizontal transfer of ARGs between bacteria is an important factor in resistance dissemination. It

is worth noting that some ARB in soil and manure are phylogenetically close to human pathogens, making genetic exchange more likely. Evidence from the last 35 years demonstrates that there was consistent correlation between the use of antibiotic-contaminated manure on farms and the transfer of ARGs in human pathogens, as well as the direct shift of ARB from animals to humans. ARGs are recognized as new environmental pollutants, and special concern is warranted due to their potential environmental and human health risks.

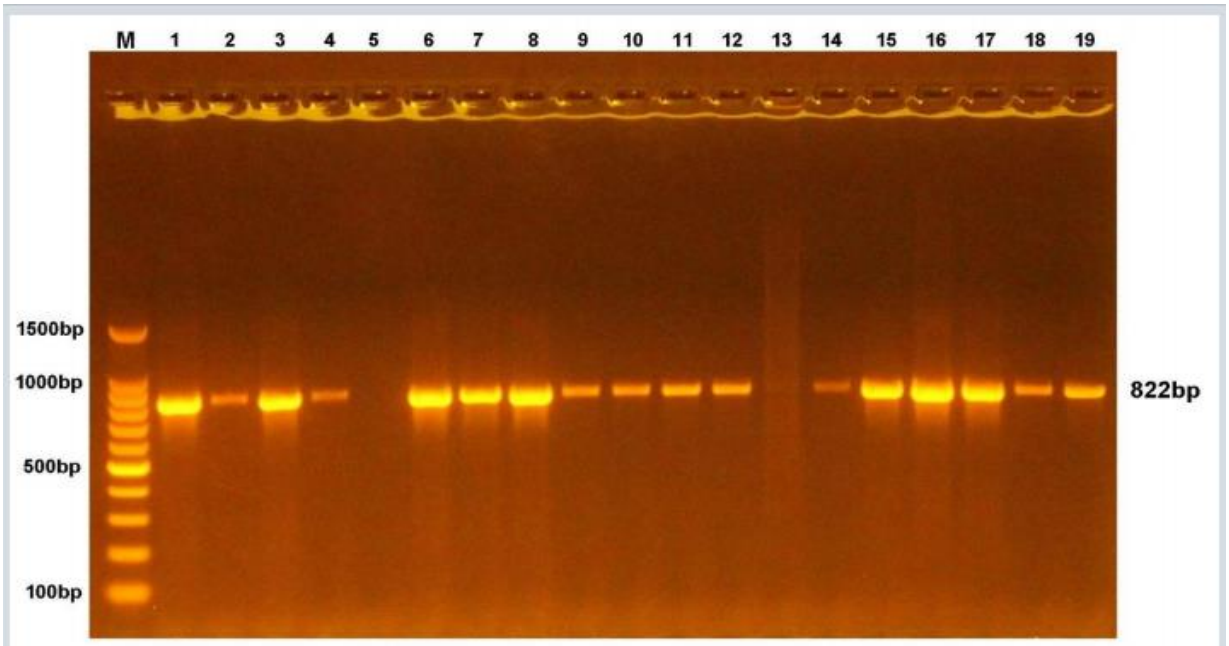

Fig.1: Gel electrophoresis of *Sul1* gene in *A. baumannii* (822 bp) at 100 volt for 75 minutes. M (Ladder 100-1500bp) (1-19) are the positive samples carrying *Sul1 gene,* (5, 13) are the negative samples.

Conclusion:

A. baumannii is a genomically variable bacterium that has the ability to cause many diseases. Sulfonamides are used to treat medical cases of animals, such as bacterial pneumonia, bacterial scours, foot rot, calf diphtheria and acute mastitis. Sulfonamides are used to treat medical cases of animals, such as bacterial pneumonia, bacterial scours, foot rot, calf diphtheria and acute mastitis. We need a range of studies about *A. baumannii* and their resistance genes, *sul1*, *sul2* and *sul3* especially in our hospitals and setting health in Iraq.

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مستخلص البحث:

تعد بكتريا *Acinetobacter baumannii* من الممرضات الانتهازية واسع الانتشار وتعد من الانواع التي تشكل تهديدًا عالميًا بسبب المقاومة العالية للعديد من المضادات الحيوية. تشير العديد من الدراسات إلى التوصيف الجزيئي لجينات مقاومة السلفوناميد وهذه الجينات المرتبطة class I integrons في العديد من البكتيريا المعوية بما في ذلك البكتريا المنتجة لمضادات البيتالاكتاميز واسعه الطيب (extended spectrum b-lactamase (ESBL تعمل جينات sul1 و sul2 و sul3 للتشفير عن dihydropteroate synthases وتم وصف أكثر من 20 جينًا مختزلاً من ثنائي هيدروفولات dihydrofolate reductase (dfr) ترتبط كلتا المجموعتين من الجينات class I integrons الموجودة في البلازميدات و أو الكروموسوم البكتيري.

تم الحصول على 19 عزلة من *A. baumannii* من حالات سريرية مختلفة بما في ذلك البلغم (8) والدم (5) ومسحة الجروح (4) ومن عينات الادرار (1) ومسحة البلعوم (1).

أظهرت نتائج الحساسية لمضادات الحيوية أن عزلات *A. baumannii* كانت مقاومة لمضادات Ceftazedim و Ciprofloxacin و Cefotaxim و Cefepime و Ampicillin و Agumantin بنسبة (100) ٪ ومضاد الجنتاميسين (94.7٪) و Imipenim (68.4) ٪.

اظهرت نتائج الفحص الجزيئي لجين (*sul1*) وجود هذا الجين في 17 عزلة بنسبة (98٪) وجميع العزلات التي تمتلك الجين كانت تمتلك مقاومة متعددة للمضادات الحيوية ، من ناحية أخرى لم يتم الكشف عن وجود جين *sul1* في السلالات 5 ، 13 وكلاهما تمتلك مقاوم لـ7 مضادات حيوية وحساسين للإيميبينيم. أظهرت نتائج الترحيل الكهربائي للهلام أن الوزن الجزيئي للجين كان 822 زوج قاعده .