Plant Archives Volume 20 No. 1, 2020 pp. 3309-3314 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ANTIMICROBIAL EFFECT OF GOLD AND SILVER NANOPARTICLES ON MDR *E. COLI* AND MOLECULAR DETECTION OF AMINOGLYCOSIDE MODIFYING ENZYMES–PRODUCING MDR *E. COLI* ISOLATED FROM UTI PATIENT

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Abstract

This study was reached in laboratories of Biology Department in Faculty of Science**.** It describes the antimicrobial effect of gold and silver nanoparticles on resistance of antibiotics bacterial species that isolated from urinary tract infection patients in the province of Najaf. A total number of 120 samples were assembled from patients with UTI admitted to AL-Sader Teaching Hospital, AL-Hakim General Hospital and AL-Furat Alawsat Hospital in AL-Najaf Governorate. The results proved the antibacterial activities of some resistance antibiotics involved Amoxicillin-clavulanic acid, Ceftriaxone, Nitrofurantoin, Cefotaxime, Ciprofloxacin and Trimethprim +Sulamethoxazole which increased in the attendance of gold with silver nanoparticles and these antibiotic together causing the inhibition of *E. coli* isolates growth and meaningfully compared with the presence of silver nanoparticles alone as well as more than the case of the presence of gold nanoparticles alone with these antibiotic. The results presented that the *aac(6')-Ib* was considered the most common types of phosphotransferase (APHs) was detected in *E. coli* isolates, 19 (98.3%) were have *aac(6')-Ib* gene and 4 (20%) were have *aph(3')-IIa* gene, while the *aac(3')-I* and *aac(3)-III* genes were not detected in any *E. coli* isolates.

Key words : nanoparticles, inhibition, silver.

Introduction

The third most common infection after respiratory and gastrointestinal infections, are Urinary tract infections. It causes a significant morbidity and considerable humanity that affects about 150 million people each year worldwide (Karve, S. *et al*., 2018). Antibiotic resistance was reported to occur when a drug loses its ability to inhibit bacterial growth effectively, bacteria become 'resistant' and continue to multiply in the presence of therapeutic levels of the antibiotics are usually effective against them, but when the microbes become less sensitive or resistant, it requires a higher than the normal concentration of the same drug to have an effect (Rahman *et al*., 2017). Due to the antibiotic resistance developed by the bacterial species that causes of the urinary tract infection, therefore, it became necessary to search for suitable alternatives to kill these species, gold and silver nanoparticles have been reported to have antimicrobial activity against a wide range of microorganism, the use of gold and silver nanoparticles antimicrobial effects is highly sought after because of its broad spectrum activity and high rate of effectiveness **(**Vieaud *et al*., 2018).

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Materials and Methods

Specimens collection and bacterial identification

A total number of 120 (urine samples) were collected from patients with Urinary tract infection admitted to AL-Sader Teaching Hospital, AL-Hakim General Hospital and AL-Furat Alawsat Hospital in AL-Najaf Governorate, during the period from October, 2019 to February, 2020. The specimens were transported by sterile transport swabs to the department of bacteriology laboratory. Each specimen was inoculated using direct method of inoculation on culture of selective media namely MacConkey, Blood, Mannitol agar then inoculated at 37°C for 18-24 hours (Chessbrough, M., 2010).

Gold and silver nanoparticles as antibacterial agent

• Preparation of gold nanoparticles : according to (Mohamed *et al*., 2017).

Table 1: Primers used in this study.

Type of enzyme	Target	Primer Name	Primer sequence	Product(pb)	Reference
ACCs	aac(6')-Ib	aac(6')-Ib-F	TTG CGA TGC TCT ATG AGT GGC TA	482	Hu *et al*., 2013
		aac(6')-Ib-R	CTC GAA TGC CTG GCG TGT TT		
APHs	aph(3')-IIa	aph(3')-IIa-F	CCT TGG TGA TAA CGG CAA TC	680	Zou *et al*., 2014
		aph(3')-IIa-R	CCA ATC GCA GAT AGA AGGC		

• Preparation of silver nanoparticles : according to (Devi *et al*., 2012).

Evaluation of the gold nanoparticles efficiency in the inhibition of the growth of antibiotic resistance bacteria that causes disease (Mohamed *et al*., 2017)

The preparation of Muller Hinton Agar, it is sterilized in the autoclave and poured in petri dishes, then antibiotic resistance bacteria were streaked by sterile swab on petri dish, antibacterial activity of the gold nanoparticles was determined using the agar well diffusion assay method, all the dishes were incubated at a temperature 37°C for 24 h. and the plates were examined for evidence of zone of inhibition, which appear as a clear area around the wells, the diameter of inhibition zones was measured using a meter ruler.

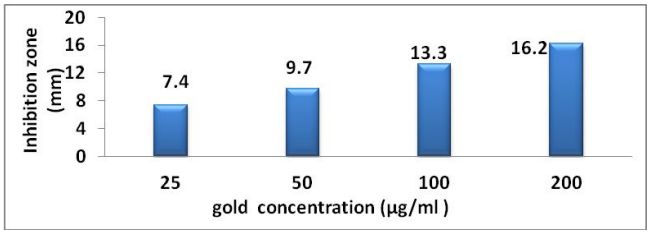


Fig. 1: Effect of different concentrations of gold nanoparticles in *E. coli* growth.

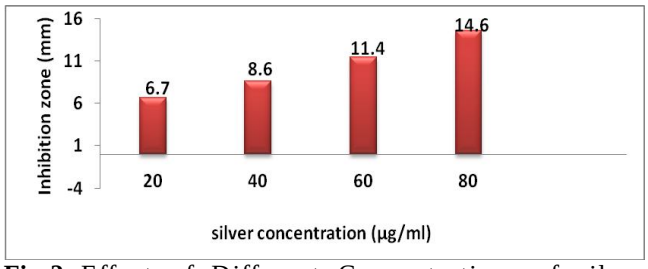


Fig. 2: Effect of Different Concentrations of silver nanoparticles in *E. coli* growth.

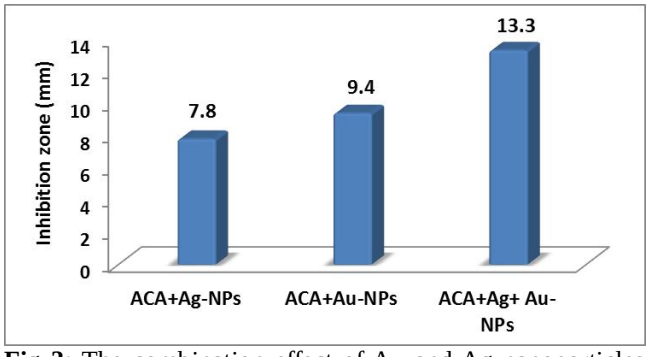


Fig. 3: The combination effect of Au and Ag nanoparticles with Amoxicillin-clavulanic acid (ACA).

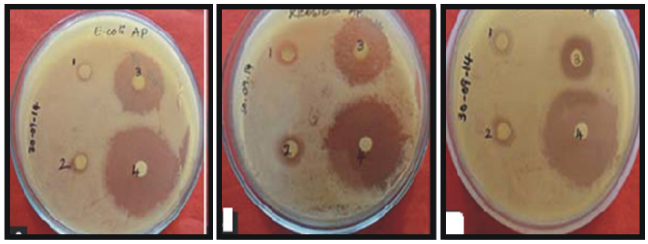
Evaluation of the silver nanoparticles efficiency in the inhibition of the growth of antibiotic resistance bacteria that causes disease (Devi *et al*., 2012)

This test is agreed in the same manner described in paragraph excluding the use of silver nanoparticles.

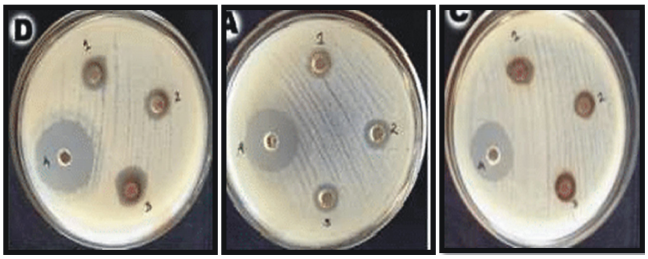
DNA Extraction

Genomic DNA was extracted by using a commercial extraction system (Genomic DNA promega Kit).

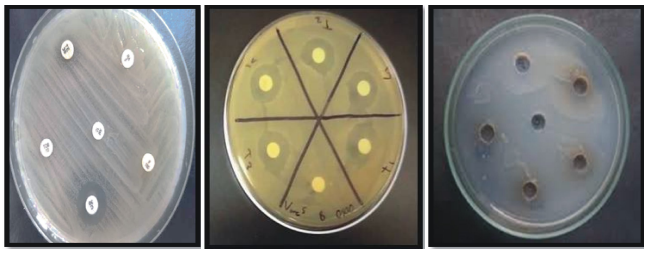
• Molecular Identification: Gel electrophoresis was used for detection of DNA by UV transilluminator. The PCR assay was performed to detect the antibiotic resistance gene for *E-coli* shown in table 2. This primer was designed by Alpha DNA company, Canada as in table 1. A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products (Levy *et al*., 2008).



Pic. 1: Effect of different concentrations of gold nanoparticles on some resistance antibiotics isolates of *E. coli.*



Pic. 2: Effect of different concentrations of silver nanoparticles on some resistance antibiotics isolates of *E. coli*



Pic. 3: A. The resistance antibiotics alone; B. The combination effect of gold nanoparticles with antibiotics; C. The combination effect of silver nanoparticles with antibiotics.

Table 2: PCR program of *AME* primer that apply in the thermocycler.

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
acc(6')Ib	95ºC for1 min	34	94ºC for 45Sec	55ºC for 45Sec	72ºC for 45 min	72ºC for 5 min
aph(3')-*lla*	94ºC for 6 min	35	94ºC for 50Sec	56ºC for 50Sec	72ºC for 80min	72ºC for 10 min

Results and Discussion

The effect of different concentrations of gold nanoparticles in antibiotics resistance *E. coli* isolates growth

The results showed that inhibition zone of *E. coli* increased progressively with increase the gold nanoparticles concentrations in reaching a maximum inhibition in 200 µg/ml fig. 1 and pic. 1. Gold nanoparticles were known to have strong antimicrobial activities the antibacterial activity of gold nanoparticles demonstrated that both gram positive and gram negative bacteria were inhibited. At the same time, they proved to be less toxic to mammal cells.

The effect of different concentrations of silver nanoparticles in antibiotics resistance *E. coli* isolates growth

The results also indicated that inhibition zone of *E. coli* increased progressively with increase the silver nanoparticles concentrations in reaching a maximum inhibition in 80 µg/ml fig. 2 and pic. 2. A lot of science reports suggests that the antibacterial mode of action of silver nanoparticles is similar to the antimicrobial effects of silver ions, due to the life cycle of silver nanoparticles and their transformation to silver ions (Xiu *et al*., 2012).

Silver ions can bond to the specialized carrier proteins and enzymes residing in the bacterial cell membrane, this chain of proteins and enzymes transport electrons and simultaneously move protons from the cytoplasm into the periplasmic space creating a concentration gradient termed the PMF, this electron transport system over the cell membrane is the primary generator of ATP during aerobic respiration in bacteria and the process is termed chemiosmosis (Nithya *et al*., 2012).

The combination effect of gold nanoparticles with most resistance antibiotics and silver nanoparticles with the same antibiotics

The results proved that the antibacterial activities of most resistance antibiotics were include Amoxicillin-clavulanic acid, Cefotaxime, Ceftriaxone, Ciprofloxacin, Trimethprim + Sulamethoxazole and Nitrofurantoin

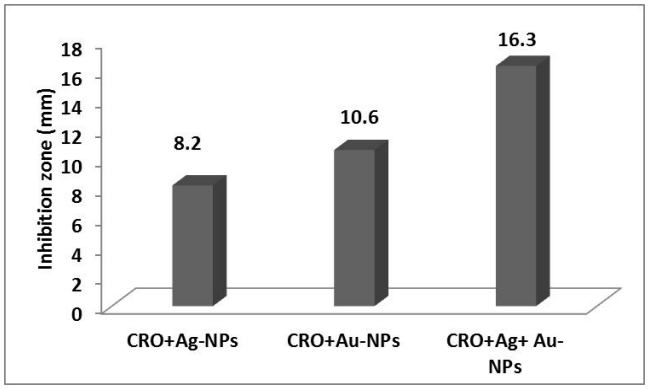


Fig. 4: The combination effect of Au and Ag nanoparticles with Ceftriaxone (CRO).

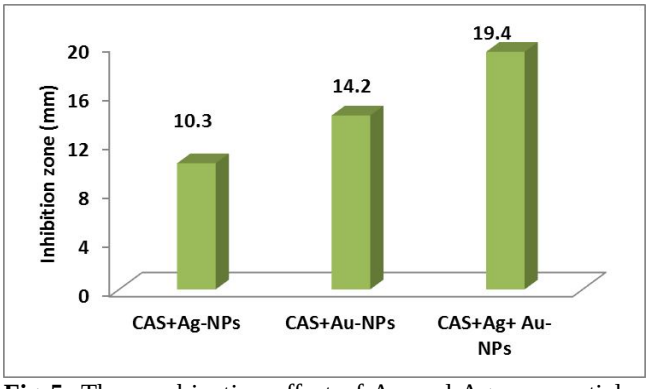


Fig. 5: The combination effect of Au and Ag nanoparticles with Cefotaxime (CAS).

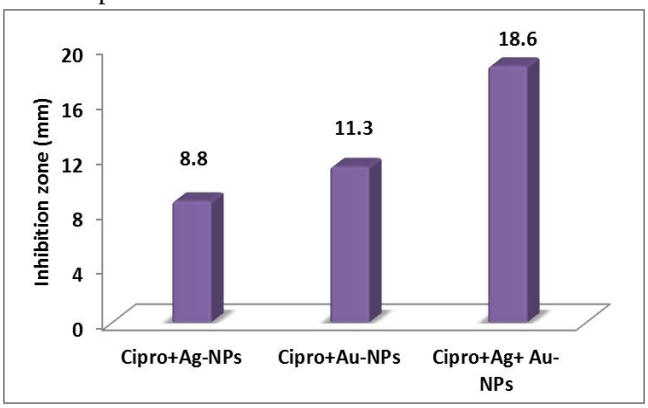


Fig. 6: The combination effect of Au and Ag nanoparticles with Ciprofloxacin (cipro).

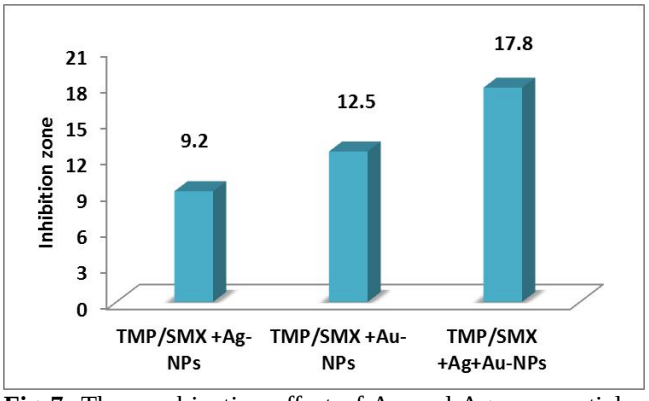


Fig. 7: The combination effect of Au and Ag nanoparticles with Trimethprim +Sulamethoxazole (TMP/SMX).

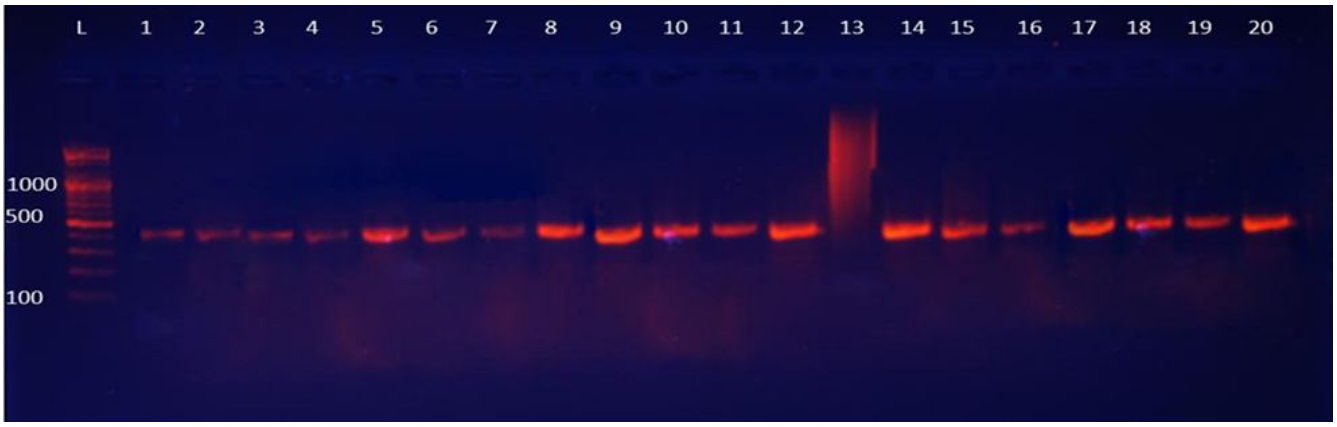


Fig. 8: Agarose gel with ethidim bromoide stained of mono-plex amplified product from extract DNA of *E. coli* isolates with *aac(6')-Ib* genes primers. Lane (L), DNA molecular size marker (100-bp ladder). Lane (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 1, 19, 20), show positive results *aac(6')-Ib* gene.

increased in the presence of gold nanoparticles as well as silver nanoparticles to causes the inhibition of multi-resistance isolates *E. coli* growth pic. 3. Although,many of these antibiotics are used very effectively towards the bacterial pathogens of urinary tract infection especially *E.coli*, but the possession of *E. coli* for many ways to resist these antibiotics, recently these ways become sophisticated significantly to resist these antibiotics resulting in not give these antibiotics any effectiveness against the *E. coli* alone, because of that many researchers are interested in using other inorganic nanoparticles as antibacterial agents, especially since gold and silver in minute concentrations are have strong antimicrobial effects (Shamaila *et al*., 2016).

Conjugates of gold nanoparticles (Au-NPs) with antibiotics have also been used for killing of bacteria because these nanoparticles have acted as a carrier for these antibiotics, Au-NPs possess a large surface to volume ratio; because of this large surface area, more antibiotic molecules get adsorbed on gold surfaces, the gold particle surrounded by a number of antibiotic moieties now acts as a single group against the microorganisms, these studies verified that Au-NPs acted as an effective carrier or anchor to these antibiotics (Pissuwan *et al*., (2010). Silver nanoparticles (Ag-NPs) have also been studied due to their inhibitory and bactericidal effects, silver antimicrobial mechanisms may include modifications of sulfhydryl-containing biomolecules such as proteins, the electrochemical collapse gradients across the bacterial cell membranes and the generation of reactive oxygen species.

The same results which showed by (Savi *et al*., 2013). when they found that the highest increase in inhibition zone in the presence of Ag-NPs was observed for vancomycin, amoxicillin and penicillin G against *E. coli, P. aeruginosa and S. aureus*. In another study, it was verified that the antibacterial activities of ampicillin, kanamycin, erythromycin and chloramphenicol were

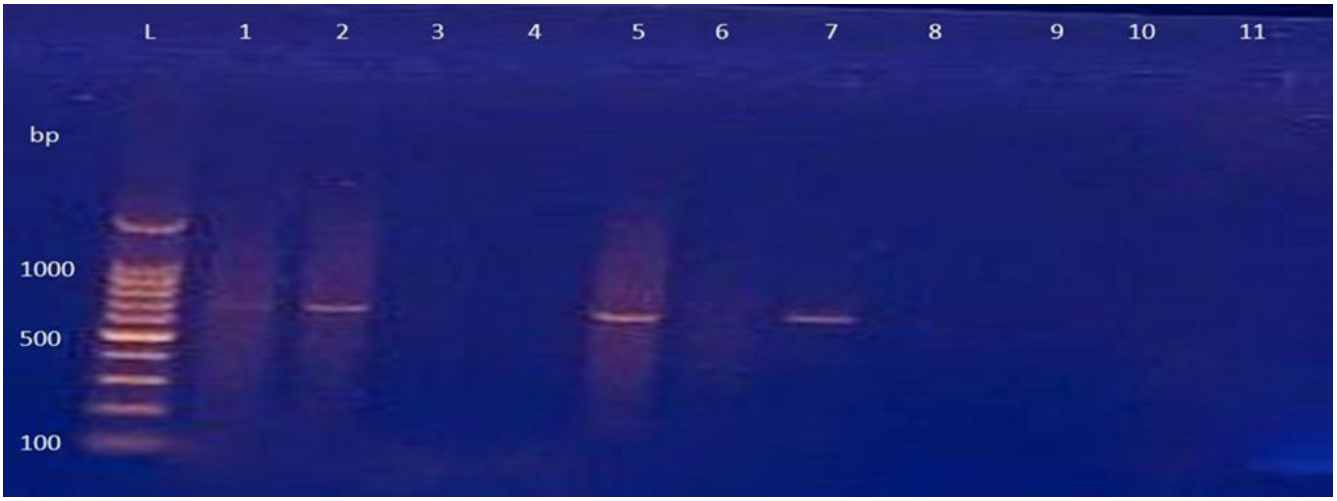


Fig. 9: Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *E. coli* isolates with *aph(3')-IIa* genes primers. Lane (L), DNA molecular size marker (100-bp ladder). Lane (1, 2, 5, 7), show positive results with *aph(3')-IIa* genes (680bp).

increased in the presence of Ag-NPs against *S. aureus* and *E. coli*, there was a less significant effect on growth of the inhibition zone on gram-positive bacteria, such as *S. aureus*, than on gram-negative bacteria, such as *E. coli* (Fayaz *et al*., 2010).

The combination effect of gold and silver nanoparticles together with most resistance antibiotics

The results showed that the presence of gold with silver nanoparticles together with the resistance antibiotic may have stimulated these antibiotics to penetrate the bacterial cell to reach their target resulting in the occurrence of inhibition zone of *E.coli* and significantly compared with the presence of silver nanoparticles alone as well as more than the case of the presence of gold nanoparticles alone with these antibiotic fig. 3 to 9. The Au-Ag nanoparticles, which are positively charged, aggregate on negatively charged bacterial cell walls, they release silver nanoparticles and generate reactive oxygen species, which are antibacterial agents, the development of bimetallic Au and Ag core–shell nanoparticles (NPs) where gold nanoparticles (Au NPs) served as the seeds for continuous deposition of silver atoms on its surface, the core–shell NPs attached to the bacterial surface and caused membrane damage leading to cell death. The enhanced antibacterial properties of Au and Ag core–shell NPs was possibly due to the more active silver atoms in the shell surrounding gold core due to high surface free energy of the surface Ag atoms owing to shell thinness in the bimetallic NP structure, recently discovered that gold-silver (Au-Ag) nanoparticles can be used to image and provide concurrent treatment for bacterial infections.

Detection of genes that responsible for aminoglycoside-resistance

The results showed that the *aac(6')-Ib* was considered the most common types of phosphotransferase (APHs) was detected in *E. coli* isolates, from the 20 (100%) isolates of *E. coli* 19 (98.3%) were have *aac(6')-Ib* gene and 4 (20%) were have *aph(3')-IIa* gene. Resistance to aminoglycosides is often due to the production of AMEs aminoglycoside modifying enzymes, AME-genes that encode the aminoglycosides acetyl-transferase (AAC), aminoglycoside nucleotideyl-transferase (ANT) and adenyl-transferase (AAD) enzymes. Aminoglycoside modifying enzymes usually confers resistance to specific aminoglycoside antibiotics but not all aminoglycoside. Production of AMEs is one of the most frequently occurring mechanisms of resistance to aminoglycoside among different *E.coli* populations for the past decade (Garneau-Tsodikova *et al*., 2016). Aminoglycoside 6'-N-acetyltransferase-type Ib [AAC(6')Ib] is considered the great clinical relevance ACC and confers resistance to tobramycin netilmicin, amikacin, dibekacin and kanamycin. The findings of the current study revealed that the rate of aac(6')-Ib gene are excessive in *E. coli* isolates and it is accordance with previous studies (Ojdana *et al*., 2018). When they found the acc(6')-Ib is the one of the most frequently detected gene. According to the present study the percentage of the presence of aph(3')-IIa gene 20% while in other studies suggested a low abundance of this gene in natural habitats and only rarely identified in bacterial isolates of human.

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