BIOACTIVITY OF *STREPTOMYCES* AGAINST COLISTIN RESISTANT *K. PNEUMONIAE* ISOLATED FROM DIFFERENT CLINICAL SAMPLES

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ABSTRACT:
This study was aimed to find a hopeful antibacterial product from *Streptomyces* spp. acts against colistin-resistant *Klebsiella pneumoniae* isolated from different clinical samples. Among 46 *K. pneumoniae* isolates, 4 (8.7%) isolates represented Colistin resistance using the disc diffusion method and minimum inhibitory concentration. Out of 20 soil samples collected from different gardens at Essaouira city, 18 *Streptomyces* spp. were isolated and identified. The bioactivity of each *Streptomyces* isolate was checked against antibiotic-resistant pneumococci. The result showed that only six isolates had high antibacterial activity, 3 isolates with moderate activity, 7 isolates with weak activity, and two with no activity. PCR and DNA sequencing of *16s rDNA* was done for the highest active *Streptomyces* spp. isolate, the result showed 96% identity to *Streptomyces azureus*.

Keywords: soil; ESBLs; carbapenem; *S. azureus;* new, inhibitory

مجلة العلوم الزراعية العراقية- 2025 :56 (2):767-775 الربيعي والربيعي

الفعالية الحيوية لبكتريا الستربتومايسز ضد بكتريا الكلبسية الرئوية المقاومة للكولستين المعزولة من عينات سريرية مختلفة

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المستخلص

الهدف من هذه الدراسة هو إيجاد نوع واعد من بكتريا الستربتومايسز بمنتجات جديدة مضادة للبكتيريا تعمل ضد بكتريا الكلبسية الرئوية المقاومة للكولستين المعزولة من عينات سريرية مختلفة. من بين 46 عزلة من الكلبسية الرئوية, 4 عزلات (8.7٪) اظهرت مقاومة للكولستين باستخدام طريقة الانتشار القرصي وطريقة الحد الادنى للتركيز. من بين 33 عينة تربة تم جمعها من حدائق مختلفة في مدينة الصويرة ، هناك 18 نوع من بكتريا الستربتومايسز تم عزلهم وتحديدهم. تم تحديد الفعالية الحيوية لبكتريا الستربتومايسز ضد بكتريا الكلِبْسية الرئوية المقاوكة للكوليستين باستخدام طريقة الانتشاربالحفر، وكانت النتيجة أن خمس عزلات فقط كانت ذات فعالية ضد ميكروبية عالية , 3 عزلات ذات فعالية متوسطة ,7 عزلات ذات فعالية ضعيفة وثلاث عزلات بدون فعالية. تم اجراء لعزلة الستربتومايسز ذات الفعالية الاعلى *16s rDNA* فحص انزيم البلمرة المتسلسل وفحص تسلسل الدنا لجين وأظهرت النتائج تطابقها 96٪ مع النوع.*Streptomyces azureus*

الكلمات المفتاحية: تربة *ESBLs;* ; كاربابينيم; *S. azureus* ; مثبط ;جديدة.

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INTRODUCTION

One of the most important ways to mediate antibiotic resistance is the bacterial production of inactivating enzymes, which include a wide spectrum of β-lactamases that degrade a wide range of antibiotics like penicillins, cephalosporins, and carbapenems (1, 9). *Klebsiella pneumoniae* that produce extended-spectrum β-lactamases (ESBLs) are major multidrug-resistant bacteria (29). The ESBL-producing *E. coli* and *K. pneumoniae* are the most common clinical isolates (12). ESBL-producing bacteria are beta-lactamase producers which are inhibited by beta-lactamase inhibitors and hydrolyze penicillins, narrow-spectrum cephalosporins, and monobactam (27). The importance of ESBL-producing organisms is due to their emergency to cause multidrug resistance, this is because ESBL's genes encoded by plasmids which also harbor other genes responsible for resistance to other antibiotic groups, e.g resistance to fluoroquinolone antibiotic transfer on ESBL-producing plasmids, consequently, the options are very rare to treat ESBL-producing bacteria (7, 28), so screening for ESBL activity in enterobacteriaceae isolates should be advised in the routine work (15). Carbapenem is the drug of choice for ESBL-producing organisms, which may be associated with both increased cost and the emergence of carbapenem-resistant Enterobacteriaceae (CRE). The treatment of Carbapenem-resistant Enterobacteriaceae diseases is very difficult due to the high failure rates, and healthcare costs of Carbapenem resistance (16). Carbapenem-resistant infection can be treated with colistin as a last choice of treatment. The *K. pneumoniae* resistance outbreaks to carbapenem and colistin were reported in many countries, like South Korea, Serbia, India, the United States, France, and Greece (33, 34). Colistin-resistant *K. pneumoniae* (CoRKp) is a serious and urgent case, so we tried to find an alternative drug against the CoRKp isolates from Streptomyces because Streptomyces is the biggest producer of the natural origin of antibiotics (20, 26).

MATERIALS AND METHODS

K. pneumonia collection and testing. Collection, isolation, and identification of *K. pneumonia.* The total number of isolated *K. pneumonia* was 46, were collected during the period (July 2021 to January 2022) from many Hospitals in Baghdad. Identification of *K. pneumoniae* was done by using MacConkey agar and the Enterosystem 18R test (Liofilchem\Italy).

Antibiotic susceptibility test of *K. pneumonia* single disk diffusion method

Carbapenem and Colistin resistance of *K. pneumoniae* isolates was detected by the Kirby-Bauer Single disk diffusion method (8, 30) (Trovafloxacin (TRV\10µg), Imipenem (IPM\10µg), Meropenem (MEM\10µg) and Colistin (CT\ 10µg) (Bioanalyse Turkey). The inhibition zone was determined by the Clinical Laboratory Standard Institute (15, 30). Cephalosporins (Ceftriaxone (CRO\ 10µg), Cefotaxime (CTX \10µg), Ceftazidime (CAZ\10µg) and Amoxycillin/Clavulanic acid (AMC\ 10µg) tested on Mueller-Hinton agar by double diffusion method to check the ESBLs production (19). The ESBL production was determined by observation of the Ghost shape zone of inhibition. McFarland's standard used was 0.5.

Determination of colistin-resistant *K. pneumonia* by MIC (minimum inhibitory concentration): The MIC of colistin was done using the agar dilution method with different concentrations of colistin (2 µg/ml to 32 µg/ml), and then *K. pneumoniae* inoculum was added readily onto the agar surface. Finally, plates were incubated at 37 °C for 24 h. The lowest concentration of antibiotics that fully inhibits visible growth is considered MIC breakpoint. Colistin susceptible isolates have a MIC of ≤ 4 µg/mL, while colistin-resistant isolates have a MIC of > 4 µg/Ml (22).

Bacteria *Streptomyces* spp. isolation and identification and testing.

Sample collection, isolation, and identification: Bacteria *Streptomyces* spp. were isolated and identified from soil samples using ISP2 and ISP4 media collected from different gardens at Essaouira city. Cultural identification, including colony morphology and Gram stain, was done by the International Streptomyces Project (4, 25).

Screening of *Streptomyces* isolates for bioactivity: Screening of *Streptomyces* was done by using the Well plate method. Antibiotic production medium (ISP2 broth)

cultured with plugs of *Streptomyces* cultures and incubated in a shaking incubator at 28°C, pH 7.5, and 170 rpm for 7 days. After fermentation, the supernatant separated from each isolate by centrifugation (10,000 rpm\2 min), and the O.D was measured at 600 nm for each isolate. The bioactivity of the supernatant (filtered by Millipore filter 0.45µm) screened against resistant bacteria. About 100 microliters of broth culture (McFarland standard used was 0.5) spread on the muller-Hinton agar after solidification, and 4-5 wells (6 mm) were prepared in each plate, Then each one was filled with the supernatant of the Streptomyces isolates (50-100ul). The zone of inhibition was checked after overnight incubation at 37 °C (18).

PCR and sequencing of Streptomyces isolate: Four Streptomyces isolates were selected for DNA extraction (highest activity). Cells were harvested by centrifugation (5 min, 4000× g), and washed [2× 10 mL of 10% (w/v) sucrose] as described by Al-Rubaye (3). Genomic DNA extraction and purification were done according to the manufacturer's instructions (Bioneer extraction kit). A microvolume UV Spectrophotometer (ACTGene, USA) was used to measure the DNA sample's concentration and purity. PCR was performed by a pair of primers targeting the *16S rRNA* for the *Streptomyces* spp. identification Table (1), aliquot preparation, and the PCR program condition, as represented in Table (2) and Table (3) respectively

Table 1. PCR primers targeting the *16S rRNA* gene for identification of Streptomyces species

Gene	Primer
16S rDNA	F: 5' TCACGGAGAGTTTGATCCTG 3' R: 5' GCGGCTGCTGGCACGTAGTT 3'

Table 2. Preparation of PCR reaction

Reaction component	Volume 1 ul	Final concentration
PCR Master Mix (2x)	12.5	1x
Forward primer (10um)	1	0.1-1.0 um
Reverse primer (10um)	1	0.1-1.0 um
DNA template	3	250 ng
Nuclease free water	25	-
Total volume	25	

Table 3. The program used to detect *Streptomyces* spp

Step	Temperature	Time	No. of Cycle
Initial denaturation	94°C	5min	1
Denaturation	97°C	30s	35-40
Annealing	50°C	1min	
Extension	72°C	1min	
Final extension	72 °C	7min.	1

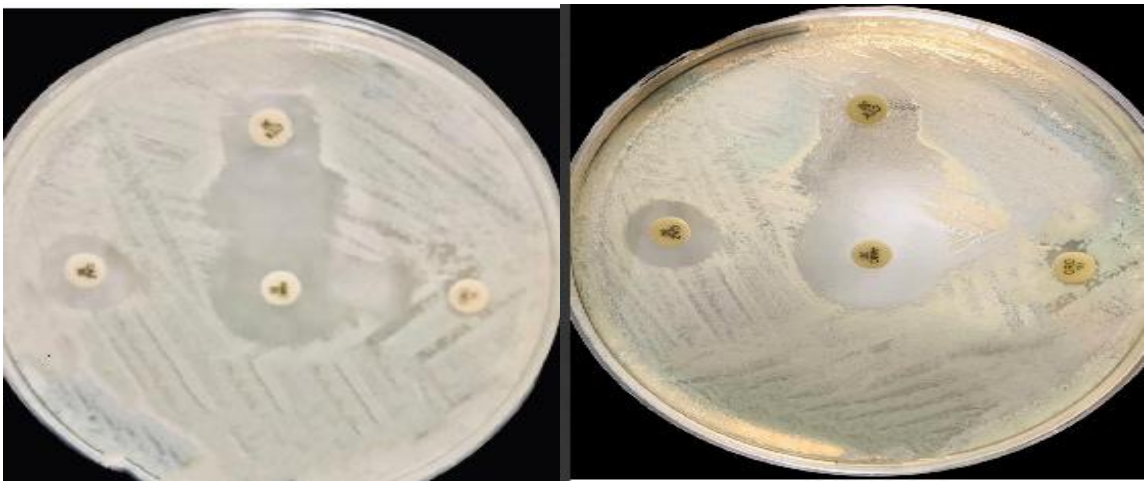
PCR products were loaded on agarose gel electrophoresis (1.5%) and visualized under UV light after staining with ethidium bromide, 5ng/ml (5). One PCR product was selected for sequencing (Bioneer, Korea), and the NCBI Blast program detected the species (ncbi.nlm.nih.gov website). A negative control was used to test the specificity of the PCR reaction.

RESULTS AND DISCUSSION

Detection of resistant *K. pneumonia*

The total number of confirmed *K. pneumonia* was 46 isolates Out of 46 *K. pneumoniae* isolates, 16 (34.8%) isolates represented ESBL positive activity by DDST (Figure 1), a ghost shape of inhibition zone referred to ESBLs positive, or the inhibition zone enhanced of any cephalosporin discs toward amoxicillin + clavulanic acid. The prevalence of carbapenem resistance among isolates was 23.9% (11\46), while the colistin resistance was 8.7 % (4\46) isolates (MIC ≥ 4 µg/ml), (Table 4). Sixteen (34.8 %) isolates with ESBL activity, while a result reported by Quan showed that the ESBL-producing *Klebsiella* was (16.5%) (31), and another report by Karki (22) showed it was 41.1%. A study by Shaikh *et al*., (32) showed that ESBL producer isolates were 55.7% *E. coli* and 44.3% *K. pneumonia,* and the ESBL-producing isolates had a co-resistance with other antibiotics, this was due to long time stay in the hospital and

previous exposure to antibiotics. Among total isolates, colistin-resistant *K. pneumoniae* were 4 (8.7 %) isolates, this result disagrees with a study by Laura (24) showed that 13% of patients with CRKp presented with ColR CRKp., also Kadum, and Al-rubaye (21) showed that the percentage of CRKp presented with ColR CRKp was 33.3%. Globally, the distribution of ESBL-producing bacteria is expanding rapidly so, control measures continuously are needed in routine work. Colistin is the last choice for Carbapenem-resistant *K. pneumonia*, so searching for another drug is an urgent and important case.



(**a**) (**b**)

Figure 1. Note the synergy between the AMC and Cefotaxime disk (CTX). DDST showed the positive results of ESBLs producing *K. pneumoniae*, using Ceftazidime disk (CAZ); Ceftriaxone (CRO) and Cefotaxime disk (CTX) disk around Amoxicillin-clavulanate (AMC) disk in the center, a is isolate (1), and b is isolate (2).

Table 4. Characteristics of colistin-resistant *K. pneumoniae* isolates

Characteristics	*K. pneumoniae*
Number of tested isolates	46
Colistin resistance determination by minimum inhibitory concentration	4
Sample types	Urine
Colistin resistance (MIC µg/ml)	One isolate = 8 µg/ml Three isolates = 16 µg/ml

Screening of *Streptomyces* spp. isolates (Bioactive compounds production)

Morphology characterization includes odor, colony morphology, the color of mycelium, colony consistency, and pigment production which is either pigment spread or localized/ Characterization by Bennett *et al*., (14), colonies showed obligate aerobic colonies with a smooth surface, then developed into aerial hyphae. The colonies are white to grayish which gives a powdered look colonies have a distinct earthy odor due to the production of a volatile metabolite called geosmin, and forming of a complex substrate mycelium with organic compounds production (17). Usually, a light microscope is used to observe the basic morphology of hyphae and spores. *Streptomyces* spp. isolates are filamentous bacteria, characterized by highly branched mycelium and Gram-positive rods with a diameter between 0.5-2.0 µm (13). Cultural characteristics suspect that the genus is Streptomyces. The microscopic characteristics show mycelium branching using Gram stain as shown in Figure (2a). Morphological characterization according to the aerial and substrate mycelium features is shown in Figure (2b). Out of 20 soil samples collected from different gardens at Essaouira city, 18 isolates were identified as *Streptomyces* spp.

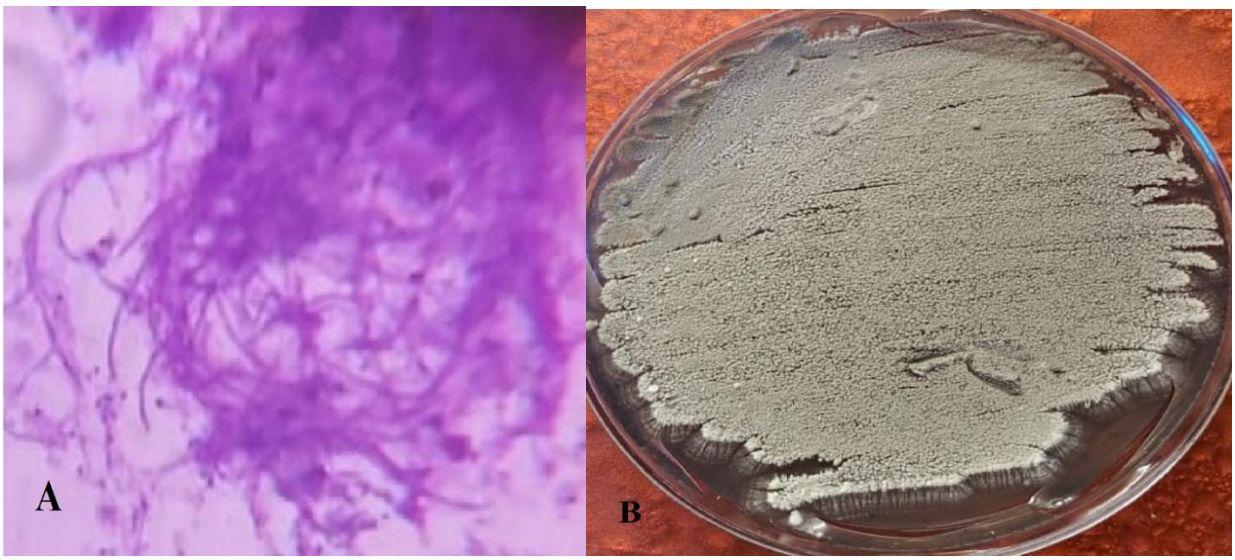


Figure 2. Streptomyces under the light microscope showing branched hyphae (A). Arial mycelium of Streptomyces grown in ISP4 (B).

Well diffusion method was used to detect the bioactivity of each *Streptomyces* (supernatant filtrate of 18 isolates) against colistin-resistant *K. pneumoniae*. The result represented that only six isolates had high antibacterial activity (Figure 3 C and D), 3 isolates with moderate activity (Figure 3 A and B, 7 isolates with weak activity, and two with no activity (Figure 3 C and D). A study by Balasubramanian and his colleagues (11), reported that the Carbapenemase-producing *K. pneumoniae* was demonstrated at a high prevalence in *K. pneumonia* isolates from pus samples, and the infection was treated by a natural antibacterial compound produced by Streptomyces. Few studies used the bioactive compounds extracted from Streptomyces against colistin-resistant *K. pneumonia* (2). The relationship between the O.D of fermentation filtrate of 18 *Streptomyces* isolates and inhibition zone diameter was measured and summarized in Table 5. Many studies use different compounds and materials other than secondary metabolites like nanoparticles to treat the global problem of antibacterial resistance (6).

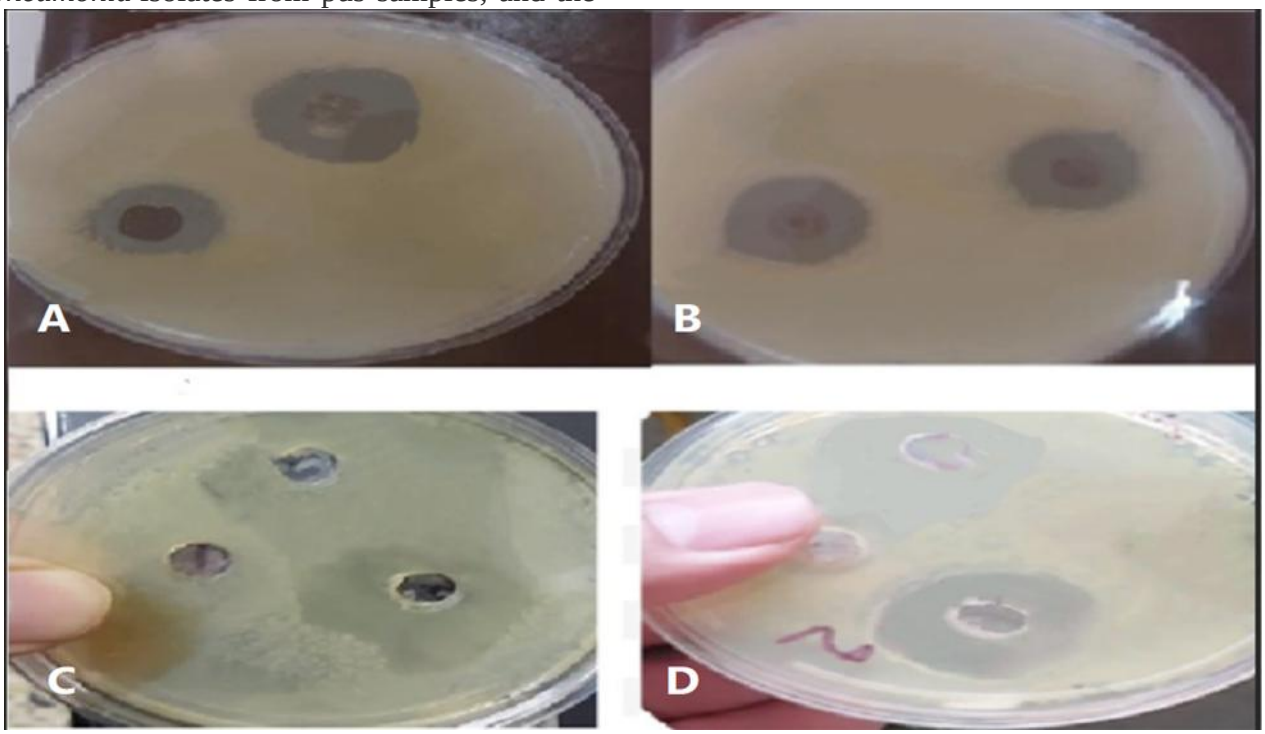


Figure 3. Well diffusion method showing zone of inhibition for Streptomyces bioactive compounds against Colistin-resistant *K. pneumonia.* (A, B, C, and D are different colistin-resistant *K. pneumonia* isolates).

Table 5. The diameter of inhibition zones by *Streptomyces* filtrates against colistin resistant *Klebsiella pneumonia* in relation to the O.D.

Degree of activity	mean of O.D (600nm)	mean of Inhibition zone (mm)
High	**1.2**	**24**
Moderate	**0.1**	**21**
Weak	**0.08**	**17**
No	**0.03**	**0**

PCR and DNA sequencing for *Streptomyces* spp. : Partial amplification of *16S rDNA* of one selected isolate is represented in Figure (4), and the sequence alignment showed similarity to the species *Streptomyces azureus* (Figure 5). This result is in agreement with studies that used *16S rDNA* for species confirmation. Partial amplification of *16S rDNA* and then sequencing is a protocol used to confirm and identify any bacteria in addition to biochemical tests (3, 4). Bacteria *S. azureus* is the producer of the thiostrepton antibiotic, it inhibits protein synthesis by binding to the protein complex (L-11) and *23s rRNA* (35), and its antibacterial properties were described by Bailly (10). Thiostrepton has activity against breast cancer cells as reported by Kwok *et al*., (23), also it is used in molecular biology as a reagent. In this instance and according to the importance of this bacteria, the recommendation is needed for the discovery of new and novel antibiotics through the designing of a local fermenter to study the production of the bioactive compounds, understand their structural and chemical nature, optimization methods for products, study in advance for the genes responsible for antibiotic production and evaluation of these products to treat the urgent resistant infection like that in *K. pneumoniae* bacteria which is considered a serious problem because it is opportunistic and causes serious nosocomial infections like pneumonia, sepsis, and urinary tract infections, and the most important consideration, in this case, is that most genes of resistance transfer by a plasmid, so treatment of such resistant bacteria prevent this transmission and prevent the development of the serious cases with MDR. Hence it is strongly recommended for the analysis of CRKp in Iraqi hospitals.

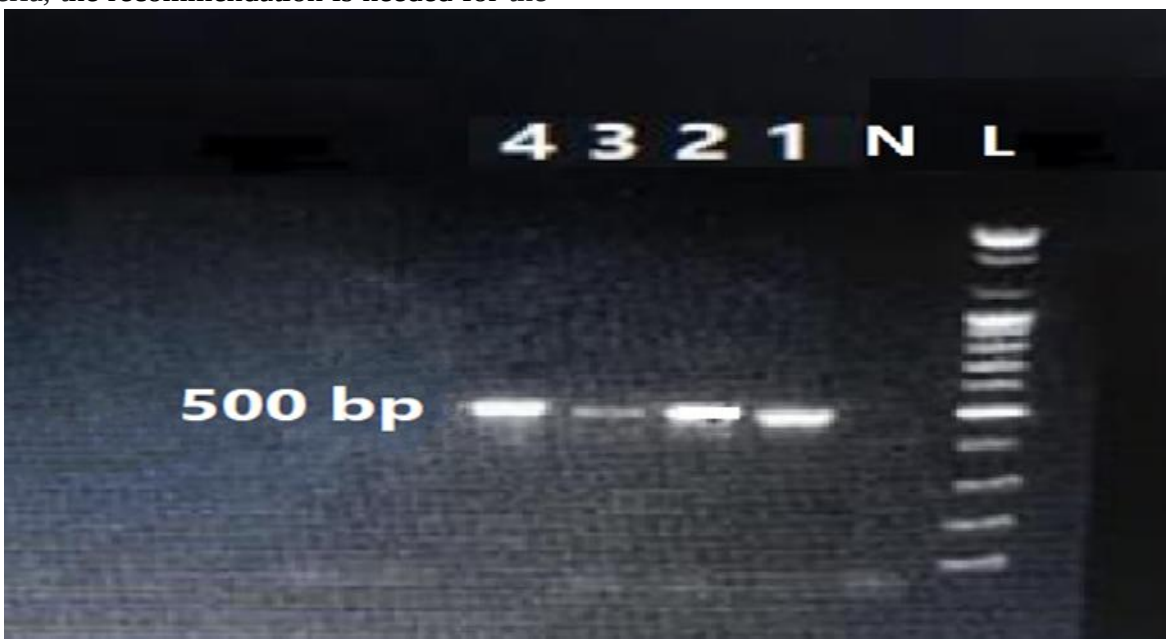


Figure 4. Agarose gel electrophoresis of *16S rDNA* partial amplification for Streptomyces isolates (agarose gel: 1.5%, 90V for 1hr. in 1x TBE buffer), stained with ethidium bromide and visualized under transilluminator UV . Lane (1,2,3 and 4): Samples, lane L: 100 bp DNA ladder and lane N: Negative control

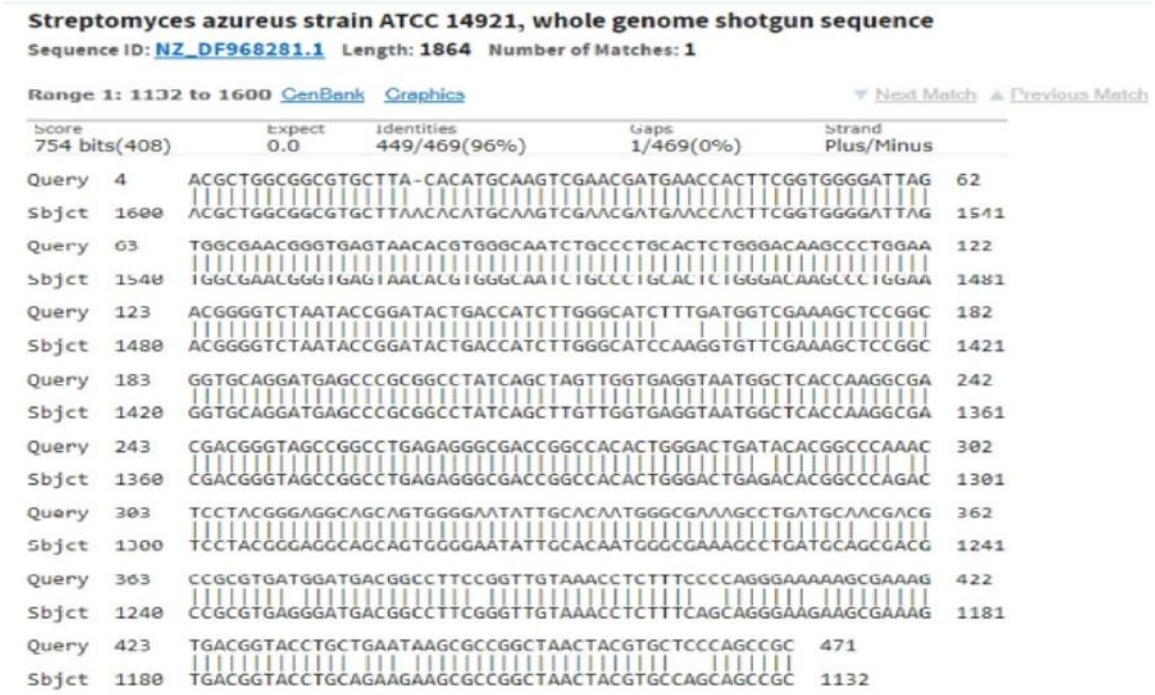


Figure 5. *16S rDNA* sequence producing significant alignment

In conclusion, this study shows that Streptomyces is a good and natural source of new antibiotics after the observation of antibacterial activity against resistant isolates, especially ESBL producers, CRKp, and CORKp.

REFERENCES

1. Al-Hasani, H. H. M.; Al-Rubaye, D. S.; Alyaa, A. (2024). Prevalence of Extended-Spectrum ß-Lactamases (ESBLs) and AmpC ß-Lactamases in Clinical Isolates of Multiple Drug-Resistant *Escherichia coli*. Iraqi Journal of Science, *65*(7), 3701-3715. https://doi.org/10.24996/ijs.2024.65.7.12.

2. Abdelaziz, R.; Tartor, Y. H.; Barakat, A. B.; EL-Didamony, G.; Gado, M. M.; Berbecea, A. and Radulov, H. I. 2023. Bioactive metabolites of *Streptomyces misakiensis* display broad-spectrum antimicrobial activity against multidrug-resistant bacteria and fungi. *Front. Cell. Infect. Microbiol.* 13:1162721. doi: 10.3389/fcimb.2023.1162721.

3. Al-Rubaye, D. S. 2016. Phylogenetic analysis of Streptomyces spp. Exhibited different antimicrobial activities. Iraqi Journal of Science, 57 (1B), 397-403. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/9201.

4. Al-rubaye, S. T.; H. M. Risan; D. Al-rubaye and R. Omran 2018. Identification and in vitro antimicrobial activity of marine *Streptomyces* spp. bacteria from Tigris River sediments in Baghdad city. World Journal of Pharmaceutical and Life Sciences. 4 (10): 120-134. https://www.wjpls.org/admin/assets/article_issue/31092018/1538631560.pdf.

5. Al-Sajad M. S., and H.A.A. Alsalim. 2024. Detection of genes responsible for heavy metals resistance in locally isolated *pseudomonas* spp. Iraqi Journal of Agricultural Sciences, *55*(1), 361-370. **https://doi.org/10.36103/wgz9vb91**

6. AL-Tameemi, A. I.; M. J.Masarudin,; R.A. Rahim, V. Timms, B. Neilan, and N.M. Isa. 2023. Antibacterial properties of zinc oxide nanoparticles synthesized by the supernatant of weissella confusa upm22mt04. Iraqi Journal of Agricultural Sciences, 54(5), 1209- 1222. **https://doi.org/10.36103/ijas.v54i5.1816**

7. Alyamani, E. J.; A.M. Khiyami; R.Y. Booq; M. A. Majrashi; F. S. Bahwerth and E. Rechkina 2017. The occurrence of ESBL-producing *Escherichia coli* carrying aminoglycoside resistance genes in urinary tract infections in Saudi Arabia. Ann Clin Microbiol Antimicrob.16 (1):1. Doi: 10.1186/s12941-016-0177-6.

8. Arciola, C. R.; D. Campoccia; Y. H. An; L. Baldassam; V. Pirini; M. E. Donati; F. Pegreddi and L. Montanaro. 2006. Prevalence and antibiotic resistance of 15 minor Staphylococcal species colonizing orthopedic

implants. The International Journal of Artificial Organs. 29 (4):395-401. Doi:10.1177/039139880602900409.

9. ATIF, M.; D.S.AL-Rubaye, and H. R. Al-Hraishawi. 2023. Plasmid profiling of extended spectrum β-lactamases producing escherichia coli in some hospitals in Baghdad. Iraqi Journal of Agricultural Sciences, *54*(2), 360-368.
https://doi.org/10.36103/ijas.v54i2.1710

10. Bailly C. 2022. The bacterial thiopeptide thiostrepton. An update of its mode of action, pharmacological properties, and applications. European Journal of Pharmacology. 5; 914:174661. Doi: 10.1016/j.ejphar.2021.174661.

11. Balasubramanian, B.; N. Benit; P. Agastian; K.S. Almaary; T.M. Dawoud; Y.B. Elbadawi; A. Mubarak; M.S. Alfadul and R. M. Aljowaie 2021. Carbapenemases producing *Klebsiella pneumoniae* from the pus of hospitalized patients: In-vitro antibiotic properties of Streptomyces against multidrug-resistant infectious bacteria. Journal of Infection and Public Health. 14 (7):892-897. DOI: 10.1016/j.jiph.2021.05.006.

12. Barrios, H.; U. Garza-Ramos; I. Mejia-Miranda; F. Reyna-Flores; A. Sánchez-Pérez; D. Mosqueda-García and J. Silva-Sanchez 2017. Bacterial resistance consortium. ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*: The most prevalent clinical isolates obtained between 2005 and 2012 in Mexico. J Glob Antimicrob Resist. 10:243-246.
DOI: 10.1016/j.jgar.2017.06.008

13. Bassam, Q. and M. Risan. 2021. Screening of bacteria *Streptomyces waksman* and henrici 1943 (Streptomycetaceae) isolates from soil samples in Iraq. Inter. J Adv Biolol and Biomed Res .9 (4): 340-351. https://doi.org/10.22034/ijabbr.2021.534456.1364.

14. Bennett, J.A.; G.V. Kandell; S. G. Kirk and J. R. McCormick 2018. Visual and microscopic evaluation of Streptomyces developmental mutants. J Vis Exp. 12 (139):57373. Doi: 10.3791/57373.

15. Clinical and Laboratory Standard Institute, Supplement, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2018. M100-S28 Performance Standards for Antimicrobial Susceptibility Testing: 28th ed. https://www.scirp.org/reference/referencespapers?referenceid=3501341.

16. Daniel, T. A.; A. Benjamin; K. A. Jones; Jesse; T. J. Mary; E.S. Zanthia; C. D. William; L. Benjamin and S. Sujit. 2022. Efficacy of noncarbapenem β-Lactams compared to carbapenems for extended-spectrum β-Lactamase -producing enterobacterales urinary tract infections, Open Forum Infect Dis, 9 (3): ofac034.
DOI: 10.1093/ofid/ofac034.

17. Deshmukh, M. B. and K. R. Sridhar. 2002. Distribution and antimicrobial activity of +actinomycetes of a fresh water coastal stream. Asian J Microbiol Biotech Environ Sci, 4: 335–340. https://www.researchgate.net/publication/267447863_Distribution_and_antimicrobial_activity_of_actinomycetes_of_a_freshwater_coastal_stream.

18. Dhanasekaran, D.; N. Thajuddin and A. Panneerselvam. 2009. Distribution and ecobiology of antagonistic Streptomycetes from agriculture and coastal soil in tamil nadu, India. J. Cult Collec. 6:10-20. https://www.bioline.org.br/pdf?cc09002.

19. Faiza, N.; I. Iqbal; N. Ikram; M. Shoaib; A. M. Javaid; R.T. Mehmood; A. Niazi; A. Anam and B. Ishfaq 2017. Phenotypic cofirmatory disc diffusion test (PCDDT), double disc synergy test (DDST), E-test OS diagnostic tool for detection of extended spectrum beta lactamase (ESBL) producing Uropathogens. J Appl Biotechnol Bioeng, (3): 344–49. DOI: 10.15406/jabb.2017.03.00068.

20. Kemung, H. M, L. T Tan, T. M. Khan, K. Chan, P. Pusparajah, B. Goh, and L Lee. 2018. Streptomyces as a prominent resource of future anti-MRSA drugs. Frontiers in microbiology, 9, 2221. https://doi.org/10.3389/fmicb.2018.02221 https://doi.org/10.36103/ijas.v49i5.50.

21. Kadum, S. M. and D. Al-rubaye. 2019. Colistin susceptibility in carbapenem resistant *Klebsiella pneumoniae* and their ability of biofilm formation. Iraqi Journal of Science. 2020:517-27.
DOI: https://doi.org/10.24996/ijs.2020.61.3.7

22. Karki, D.; B. Dhungel; S. Bhandari; A. Kunwar; P.R. Joshi; B. Shrestha; K. R. Rijal; P. Ghimire and MR. Banjara 2021. Antibiotic

resistance and detection of plasmid mediated colistin resistance mcr-1 gene among *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples. Gut Pathog. (5): 13 (1):45. doi: 10.1186/s13099-021-00441-5.

23. Kwok, J.M.; S.S. Myatt; C.M. Marson; R.C. Coombes; D. Constantinidou and E.W. Lam 2008. Thiostrepton selectively targets breast cancer cells through inhibition of forkhead box M1 expression. Mol. Cancer Ther. 7 (7): 2022–32.
DOI: 10.1158/1535-7163.MCT-08-0188.

24. Laura, J. R.; S. Madiha; C. Eric; S. R. Sandra; P. Federico; A. S. Robert; C. K. Robert; R. W. Richard; M. Steve; D. R. Susan; D.T. Nicholas; M. H. Andrea; M. H. Kristine; D. Yohei; S. K. Keith; E. Scott; G. F. Vance; Jr; A. B. Robert and van D. David. 2017. For the Antibacterial Resistance Leadership Group, Colistin Resistance in Carbapenem-Resistant *Klebsiella pneumoniae:* Laboratory Detection and Impact on Mortality, Clin Infec Dis, 64 (6):711–718.
https://doi.org/10.1093/cid/ciw805

25. MacFadden, J. F. 2000. Biochemical Tests for Identification of Medical Bacteria 3rd ed. The Williams and Wilkins Co., USA, 689-691.
https://lib.ugent.be/catalog/rug01:000694128

26. Maiti, P.K.; S. Das; P. Sahoo and M. Sukhendu 2020. *Streptomyces* sp SM01 isolated from Indian soil produces a novel antibiotic picolinamycin effective against multi drug resistant bacterial strains. Sci Rep. 10, 10092.
DOI:10.1038/s41598-020-66984-w.

27. Mariana, C.; J. S. Patricia and A. B. Patricia 2021. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection, JAC-antimicrobial resistance, 3 (3): dlab092. DOI: 10.1093/jacamr/dlab092.

28. Medina-Pizzali, M. L.; A. Venkatesh; M. Riveros; D. Cuicapuza; G. Salmon-Mulanovich; D. Mäusezahl and S.M. Hartinger. 2022. Whole-Genome Characterisation of ESBL-Producing *E. coli* isolated from drinking water and dog faeces from rural andean Households in Peru. Antibiotics. 11(5):692.
doi: 10.3390/antibiotics11050692.

29. Mustafa, M. G.; M. A. El-Mahdy and F. B. Rasha. 2017. Association between virulence factors and extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* compared to nonproducing isolates, Interdiscip Perspect Infect
Dis., vol. 2017, Article ID 7279830, 14 pages.
doi: 10.1155/2017/7279830.

30. Nassar, M.S.M.; W.A. Hazzah and W.M.K. Bakr. 2019. Evaluation of antibiotic susceptibility test results: how guilty a laboratory could be?. J. Egypt. Public. Health. Assoc. 94, 4. doi: 10.1186/s42506-018-0006-1.

31. Quan, J.; D. Zhao; L. Liu; Y. Chen; J. Zhou; Y. Jiang; X. Du; Z. Zhou; M. Akova and Y. Yu. 2017. High prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in community-onset bloodstream infections in China. J Antimicrob Chemother.72 (1):273-280.
doi: 10.1093/jac/dkw372.

32. Shaikh, S.; J. Fatima; S. Shakil; S.M. Rizvi and M. A. Kamal. 2015. Risk factors for acquisition of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in North-Indian hospitals. Saudi J. Biol. Sci, 22(1), 37–41. doi: 10.1016/j.sjbs.2014.05.006.

33. Sharma, S.; T. Banerjee; A. Kumar; G. Yadav and S. Basu. 2022. Extensive outbreak of colistin resistant, carbapenemase (bla_{OXA-48}, bla_{NDM}) producing *Klebsiella pneumoniae* in a large tertiary care hospital, India. Antimicrob Resist Infect Control. 6;11 (1):1. doi: 10.1186/s13756-021-01048-w.

34. Tania, N. D.; E. Azar; C.Al-Bayssari; S. C. Amanda and Jean-Marc Rolain 2019. First Detection of colistin-resistant *Klebsiella pneumoniae* in association with NDM-5 carbapenemase isolated from clinical Lebanese patients. Microb Drug Resis. pp: 925-930. doi: 10.1089/mdr.2018.0383.

35. Walter, J. D.; M. Hunter; M. Cobb; G. Traeger and P. C. Spiegel. 2012. Thiostrepton inhibits stable 70S ribosome binding and ribosome-dependent GTPase activation of elongation factor 4. Nucleic Acids Res. 40 (1):360-70. doi: 10.1093/nar/gkr623.