EMERGENCE OF MULTIDRUG RESISTANT BACTERIA AMONG PATIENTS WITH RESPIRATORY TRACT INFECTIONS

Omaima S Al-Qaissy[1] **Ahmed S. K. Al-Khafaji[1,2]**
Researcher **Assist.Prof.**
[1]Department of Biology, Collage of Science, University of Baghdad, Baghdad, Iraq
[2]Leading National Cancer Research Centre, University of Baghdad, Medical City,Bab Al Muadham, Baghdad, 10047, Iraq
E-mail: omayma.ibraheem1202@sc.uobaghdad.edu.iq E-mail: khafaji@sc.uobaghdad.edu.iq

ABSTRACT

In this study, 25 *Pseudomonas aeruginosa* isolates were diagnosed in 160 clinical samples collected from patients attending the Tuberculosis Institute/ Medical city and Al-Muhmodia Public Hospital, in addition to a number of private laboratories in Baghdad during the period from September 2020 to February 2021. The bacterial isolates from clinical samples were biochemically diagnosed by API® 20E system then further identified by VITEK 2 system. *P. aeruginosa* represented the most prevalent bacteria in lower respiratory tract specimens. These isolates showed moderate to high susceptibility towards 11 antimicrobial agents tested in this study except that of ceftazidime and ceftriaxone. Extra-chromosomal resistance of the top three of the most resistant *P. aeruginosa* isolates were subjected for molecular investigation. Bacterial transformation of *E. coli* S1 cells with plasmid DNA extracted from the selected *P. aeruginosa* isolates exhibited increased resistance to ceftazidime and penicillin G. The data clearly suggest that the plasmid DNA content is implicated in enhancement of *P. aeruginosa* resistance through horizontal transfer of extra- chromosomal beta lactam resistance associated genes.

Keywords: *Pseudomonas aeruginosa,* plasmid DNA, transformation, β-lactam resistance, horizontal gene transfer, respiratory infections.

ظهور البكتريا المقاومة للأدوية المتعددة بين مرضى التهابات الجهاز التنفسي

أميمة سعد القيسي **أحمد سالم كاظم الخفاجي**
باحث **أستاذ مساعد**
[1]قسم علوم الحياة كلية العلوم جامعة بغداد
[2] المركز الوطني الريادي لأبحاث السرطان ، جامعة بغداد ، مدينة الطب، باب المعظم ، بغداد ، 10047 ، العراق

المستلخص

تركزت هذه الدراسة على عزل وتشخيص 25 عزلة من بكتريا الزائفة الزنجارية من اصل 160 عينة من المرضى المراجعين لمعهد التدرن في مدينة الطب ومستشفى المحمودية وبعض المختبرات الخاصة في بغداد. البكتريا المنعزلة من العينات السريرية تم تشخيصها بااستخدام الاختبارات الكيمياحيوية والمجهرية و API 20 وتم تأكيد التشخيص باستخدام جهاز الفايتك. تمثل الزائفة الزنجارية اكثر العزلات انتشارا في الجهاز التنفسي السفلي. اظهرت عزلات الزائفة الزنجارية حساسية متوسطة الى عالية تجاه 11 نوع من المضادات الحيوية التي تم اختبارها في هذه الدراسة عدا السفتازديم والسيفاترايكزون. تم التحري الجزيئي عن المقومة الخارج كروموسومية في اعلى ثلاث عزلات مقاومة للمضادات الحيوية بطريقة التحول البكتيري . اظهرت نتائج التحول البكتيري للدنا البلازميدي المستخلص من العزلات الثلاثة المختارة بأن خلايا S1 للأشريشية القولونية المحولة اصبحت ذات مقاومة متزايدة للسيفتازديم والبنسلين جي. وهذه النتائج تشير بوضوح الى ان محتوى الدنا البلازميدي هو المسؤول عن تعزيز مقاومة الحيوية لصنف البيتا لاكتام عن طريق الانتقال الافقي لجينات المقاومة الخارج كروموسوية للزائفة الزنجارية.

الكلمات المفتاحية: الزائفة الزنجارية, الدنا البلازميدي, التحول, مقاومة البيتا لاكتام، النقل الجيني الافقي، الاخماج التنفسية.
Received:2/8/2021 Accepted:21/11/2021

INTRODUCTION

P. aeruginosa has been recognized as a primary opportunistic human pathogen for several decades (10) and has the potential to induce respiratory tract infections. Virulence factors and antimicrobial degrading enzymes are needed to overcome the immune system defenses resist antimicrobial treatment to initiate infection and invasion, which aids in their pathogenesis, survival, and immune system evasion (20,24). *P. aeruginosa* is a gram- negative bacilli that is non-spore forming,encapsulated, and usually motile due to the presence of one or two polar flagella (1,19). Respiratory tract infections (RTIs) representthe most common infectious diseases affecting individuals' health worldwide (30).The etiological agents of respiratory infections vary from one area to another aswell as their antibiotic susceptibility.*Streptococcus*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Haemophilus influenza* are the most common infectious bacteria ofrespiratory tract (29).

Respiratory tract infections can be caused by bacterial or viral origin, with a strong overlap in clinical signsand symptoms (27,28). Plasmid DNA is an extra-chromosomal DNA that replicates independently of the bacterial chromosome. Plasmids in nature range in molecular size from one to several hundred kilo base-pairs and in the number ofcopies per cell from one to several hundred. Under constant conditions, the copy number of any plasmid is a fixed feature (4). Horizontal gene transfer (HGT) is a major source of bacterial genome variation and evolution (26). Bacterial HGT techniques include conjugation, transduction, and natural transformation. The natural transformation involves importing bare DNA from the environment (13). On the other hand, artificial transformation techniques are the commonly employed procedure in molecular biology studies using electroporation or heat shock treatments in order to test the effect of bacterial HGT on modifying antibiotics response (25). Susceptibility profiles *of P. aeruginosa* are geographically variable, and susceptibility testing should be used in conjunction with antimicrobial therapy collection. *P.aeruginosa* is resistant to a broad spectrum of antibiotics, including aminoglycosides, β- lactams and quinolones. Thus, *P. aeruginosa* infections are rarely to be treated with single-drug treatment due to the poor success rate because the bacterial resistance develops rapidly when antimicrobial agents are administrated as mono therapy but not in combination (12). Therefore, the current study aims to investigate the impact of HGT emerging antibiotics resistance of *P. aeruginosa* infecting human respiratory tract.

MATERIALS AND METHODS

Samples collection :A total of 160 clinical samples were collected from Tuberculosis Institute and Al-Muhmodia Public Hospital in Baghdad. Samples were collected according to different parameters (age, gender, sample type, smoking status, and previous diagnosis) during the period from September 2020 to the middle of February 2021. The ethical approval of the current study had been granted by The College of Science Research Ethics Committee at the University of Baghdad under the reference number"CSEC/0421/0031". All of the clinical samples were collected autonomously from individuals who have given their informed consent.

Bacterial isolation and diagnosis : Selective conditions of growth on 0.3% Citramide agar at 42°C overnight incubation were applied for the preliminary bacterial detection of *P. aeruginosa*. The preliminary detection was confirmed by biochemical tests in accordance with microbiological protocols (6), and then was further confirmed using advanced API system (API 20E) and VITEK2 system (7).

Antimicrobial susceptibility

The antimicrobial sensitivity of diagnosed isolates was investigated using disc diffusion method. 0.1 ml of bacterial suspension (0.5 McFarland dilution) of each isolate was inoculated on Muller Hinton agar plates by spreading method. The antibiotic discs of amikacin (30µg) AK, ciprofloxacin (10µg) CIP, ceftazidime (30µg) CAZ, ceftriaxone (10µg) CRO, meropenem (10ug) MEM, cefpodoxime (30µg) CPM, imipenem (10µg) IPE, aztreonam (30µg) ATM, levofloxacin (5µg) LEV, gentamicin (10µg) GN and piperacillin-tazobactam (110µg) PTZ were applied on the agar surface and the plates were incubated for overnight at 37° C. The findings

were read and interpreted based on the Guideline of Zone Diameter Interpretive Standards for *P. aeruginosa* (3).

Plasmid DNA extraction

Plasmid DNA of *P. aeruginosa* was extracted utilising ZyppyTM Plasmid Miniprep Kit (Catalogue no. D4019) according to the manufacturer protocol. In brief, 2 ml of overnight grown bacteria was subjected to 100 µl of 7X Lysis Buffer. 350 µl of cold Neutralization Buffer was added tothe mixture and then applied to the centre of the provided Zymo-spin™ IIN column after converting the colour from blue to yellow. The column was centrifuged for 15 seconds 200 µl of Endo-Wash Buffer, then 400 ul of Zyppy™ Wash Buffer was added and centrifuged as aforementioned. The column transferred into a 1.5 ml tube, and 30 µl of Zyppy™ Elution Buffer was added then centrifuged as aforementioned to elute the plasmid DNA. The DNA concentration and purity were measured by Nanodrop 2000 Spectrophotometer.The molecular size of the plasmid DNA was estimated by agarose gel electrophoresis using AccuBand™ 100 bp+3K DNA LadderII.

Bacterial transformation with plasmidDNA

In order to explore the role of plasmid DNA in elevating antibacterial resistance, Stellar Competent *E. coli* bacterial cells s1(S1 competent cells vial was a kind gift from the Molecular and Clinical Cancer Medicine Department at the University ofLiverpool, UK. The storage temperature is - 20)(Catalogue no. C4040-03– Life technologies) were transformed with the extracted *P. aeruginosa* plasmid content based on the manufacturer protocol. Briefly, 2 µl of 50 ng/µl plasmid DNA was added intothe competent *E. coli* cells contained vial. The mixture was incubated on ice for 30 minutes. Afterward, the cells were exposed to heat-shock for 30 seconds at 42°C without shaking. The vial was re-incubated on ice for2 minutes. Subsequently, 500 µl of pre-warmed SOC broth was added to the vial, and then incubated in a shaking incubator at 37°C for 1 hour at 200 rpm. Finally, 100 µl from transformation mixture was inoculated on a pre-warmed selective medium plate (Luria-Bertani medium contained 100 µg/ml of each tested antibiotics).

Statistical analysis

Chi square tests were conducted for statistical analyses of the study results, wherep-values of less than 0.05 have been considered as significant

RESULTS AND DISCUSSION

Bacterial isolation and prevalence among the collected samples.Among the total of 160 cases, from which clinical samples were obtained, 60% (96) of the cases were undiagnosed and 40% (64) were diagnosed cases. This clinical record is expected since more of the attended individuals have no previous diagnosis history. One hundred and twenty 75% ofsamples were sputum, while 15 % (24) represented pleural fluid, and the rest 10% (16) were bronchial wash. The *P. aeruginosa* isolates grown on citramide agar at 42°C for 18 hr were smooth in shape with flat edges, fruity odour and fluorescent green colonies. On blood agar, the growing bacteria showed mucoid colonies with a typical metallic sheen. The bacterial growth properties are in agreement with Don (10) The top three of diagnosed cases represent 15% pneumonia (24 cases), 8.75% chronic bronchitis (suspected COPD) (14 cases), 7.5% old healed tuberculosis (TB) (12 cases)(Figure 1).

Cases number

Undiagnosed Chronic bronchitis (Suspected COPD)

covid-19Allergic respiratory infection

Cystic fibrosisBronchiectasis

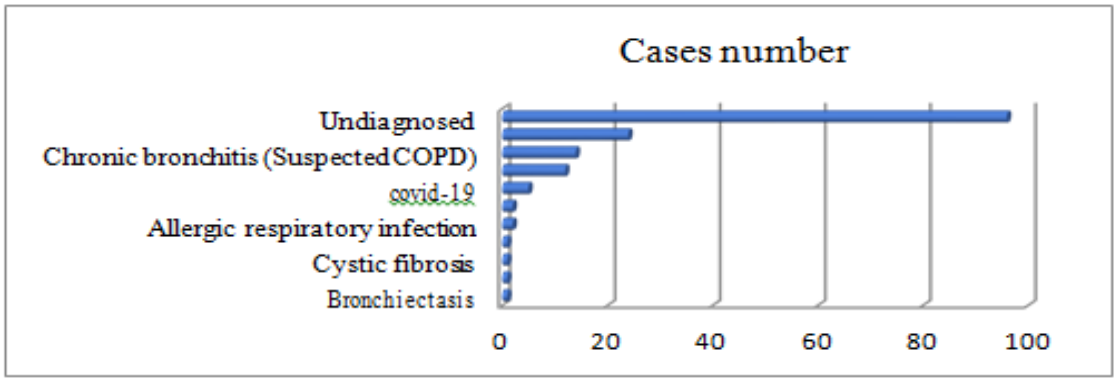

Figure 1. The bar-chart shows the distribution of the total of 160 cases, from which clinical samples were obtained, between diagnosed and undiagnosed cases. The figurealso illustrates that pneumonia, chronic bronchitis (suspected COPD), old healed tuberculosis (TB) were the most prevalent respiratory diseases of the diagnosed ones

According to the screening of the collected clinical samples, bacterial isolates were more prevalent in the sputum samples (Figure 2) compared to that in either samplesof bronchial wash (Figure 3) or pleural fluid (Figure 4). In general, the bacterial prevalence accounts for 95% (152specimens) out of the total number of the collected specimens. In sputum specimens, *P. aeruginosa* isolates represent the 2nd highest percentage 17% (20 isolates) after that of *Klebsiella pneumoniae* 19% (22 isolates) (Figure 2).

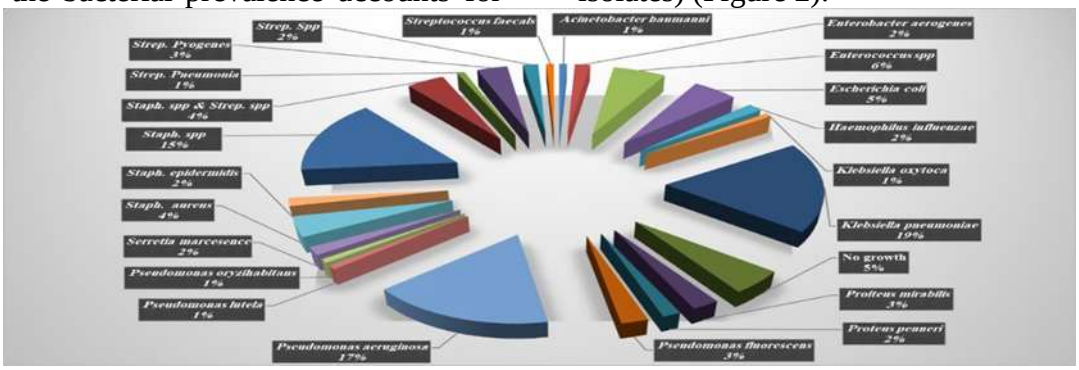

Figure 2. Bacterial prevalence in the collected sputum samples of respiratory tract

Infections: Regarding the bronchial wash samples collected from respiratory tract infections, *P. aeruginosa* isolates recorded the highest proportion of isolated bacteria accounting for 20% (3 isolates) among the clinical samples despite their limited number (15 isolates) dueto the technical difficulties of obtaining such type of samples. This could be expected sincebronchoalveolar lavage obtained from the bronchi and bronchioles may contain more *P. aeruginosa* population than upper respiratoryswaps (13). On the other hand, more than half of the samples (53%) have no any bacterial prevalence.

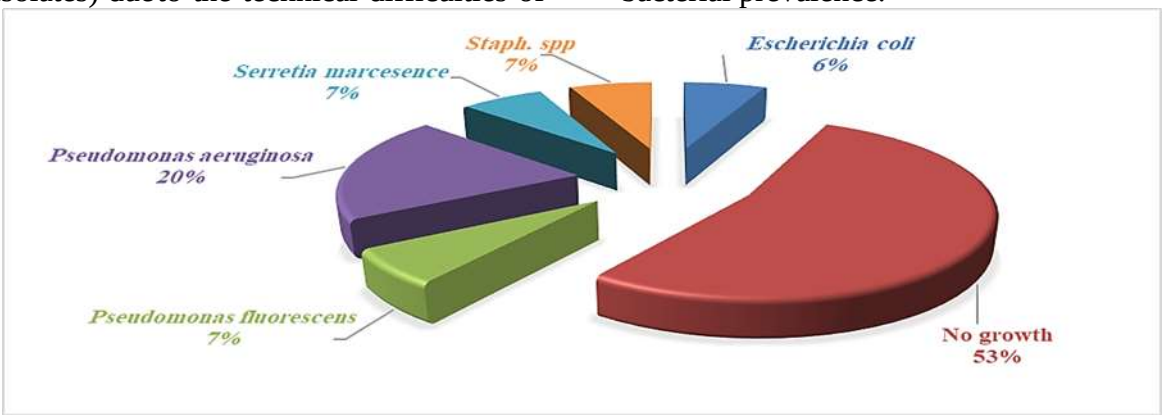

Figure 3. Bacterial prevalence in the collected Bronchial wash samples of respiratorytract infections

Likewise, *P. aeruginosa* isolates were more prevalent in the obtained pleural fluid specimens accounting for 8% (2 isolates) (Figure 4). Taken together, our bacterial screening results indicate that *P. aeruginosa* is the most prevalent bacterial pathogen among the microbial population of the respiratory tract infections. These findings are in line with previous studies that reported *P. aeruginosa* as a leading bacterial cause ofrespiratory diseases (3,10) and (19).

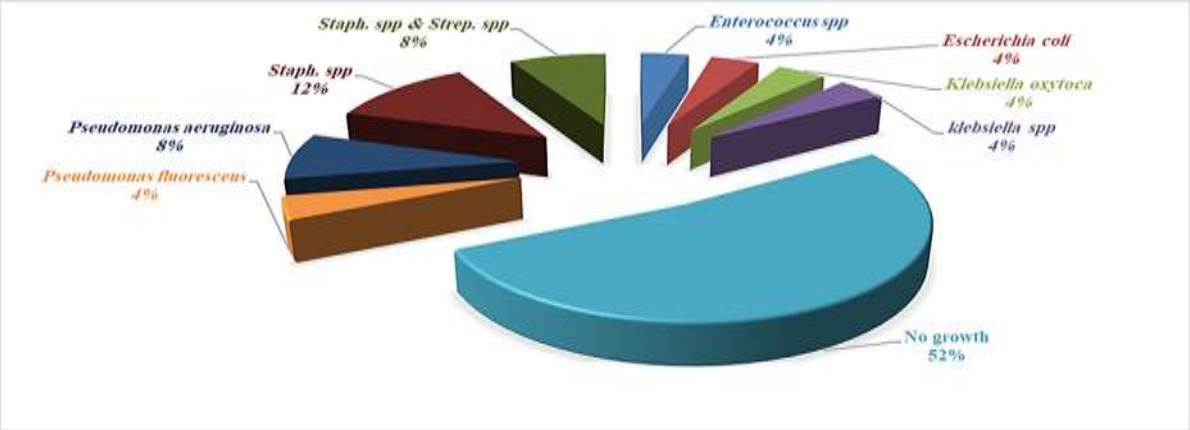

Figure 4. Bacterial prevalence in the collected Pleural fluid samples of respiratory tract Infections

Antibiotic susceptibility

The antibiotic susceptibility investigation of the diagnosed *P. aeruginosa* isolates exhibited variable responses to the examined antimicrobial agents (amikacin, ciprofloxacin, ceftazidime, ceftriaxone,meropenem, cefpodoxime, imipenem, aztreonam, levofloxacin, gentamicin and piperacillin-tazobactam. However, the common susceptibility trend demonstrates that most of the *P. aeruginosa* isolates were almost sensitive to the most tested antibiotics 81.8% except that of ceftazidime and ceftriaxone 18.2%. However, the examined isolates also showed moderate response to 27% of the tested antibiotics (ATM, LEV, CN) (Figure 5). It has been reported that emergence of ceftazidime-resistant *P. aeruginosa* strains pose a threat to the healthsystem and, therefore a combination of ceftazidime-avibactam is currently used to overcome such a medical problem (7,31) and (32).

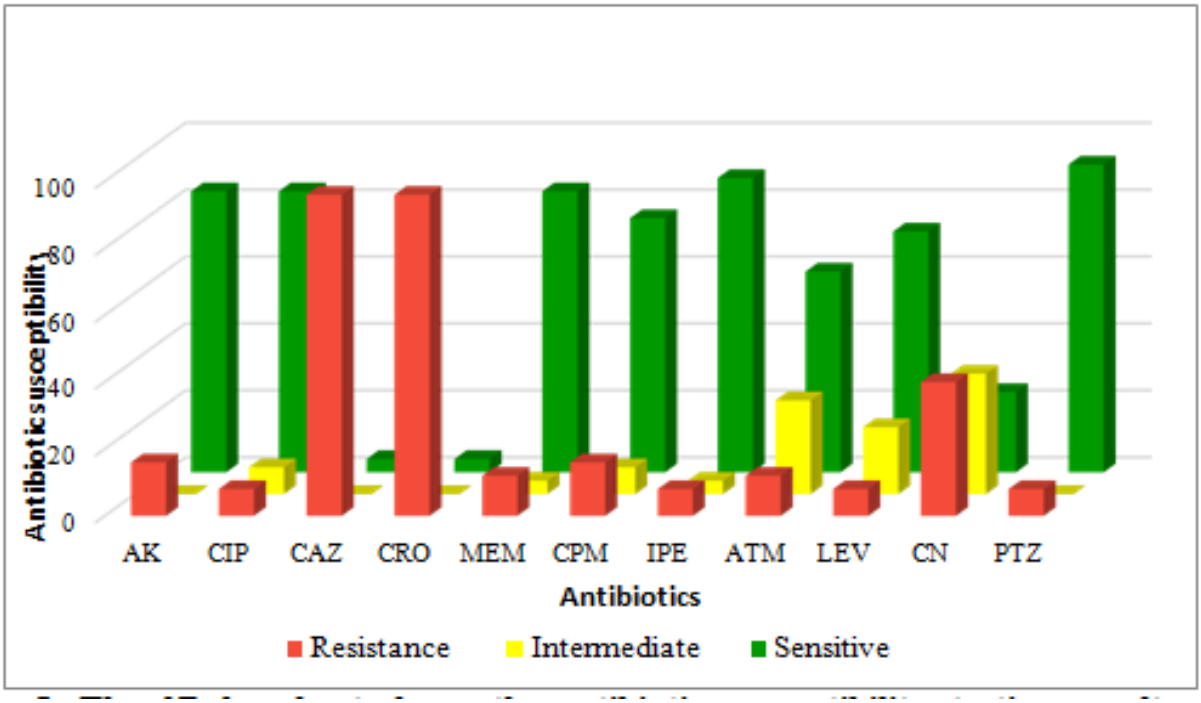

Figure 5. The 3D barchart shows the antibiotic susceptibility testing results of the examined *P. aeruginosa* isolates

The top three of the most resistant isolatesof *P. aeruginosa* were subjected for preliminary molecular analysis. The plasmid DNA content of the chosen isolates was extracted and analysed by gel electrophoresis technique. The outcome demonstrated that each of the tested bacterial isolates harbour unique set of plasmid profile (Figure 6). Each plasmid profile may reflect the different topological conformations for the same plasmid (18). Surprisingly, the calculation analysis of the linear bands of the plasmid DNA molecules reveals that the DNA molecular weight is around (1.5) kbp. This indicates the existence of lower molecular weight of such extra-chromosomal DNA than that has been reported to be harboured by *P. aeruginosa* so far. It has been recently reported that *P. aeruginosa* (3.652) kbp, which is as twice large as that we have explored in the current study (16). This result, for definite, need to be further analysed.

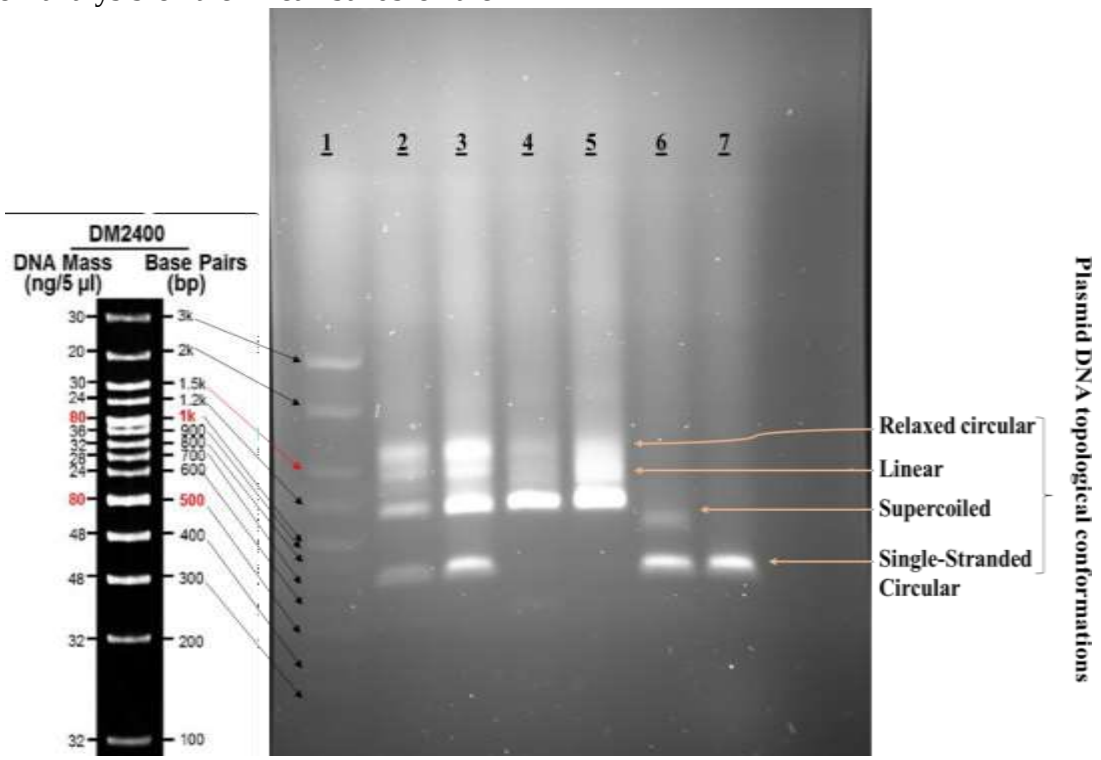

Figure 6. The image of agarose gel electrophoresis demonstrates 7 DNA migration lanes; 1) AccuBand™ 100 bp+3K DNA Ladder II, in addition to plasmid DNA profile extracted from the studied *P. aeruginosa* isolates as follows:
2, 3) the plasmid DNA content of *P. aeruginosa* PA7 symbolized as (pPA7).
4, 5) the plasmid DNA content of *P. aeruginosa* PA42 symbolized as (pPA42).
6, 7) the plasmid DNA content of *P. aeruginosa* PA51 symbolized as (pPA51).

Bacterial transformation

In order to uncover the potential role of the *P. aeruginosa* plasmid DNA content in increasing the antibiotics resistance, *E. coli* S1 competent cells have been transformed by the extracted plasmid DNA of *P. aeruginosa* isolates PA7 and PA42 and PA51, which are coded as; "pPA7, pPA42 and pPA51" respectively. The *E. coli* S1 competent cells, which are also known as Stellar^{TM} Competent Cells, represent competent *E. coli* HST08strain that had been genetically modified to eliminate all the genetic factors involved in antibiotics resistance. The results showed the successful transformants cells were able to grow on only Ceftazidime or Penicillin G containing medium in comparison to the non-transformed parental cells, as one representative colony growing out of each successful transformed cells were subjected to antibiotic sensitivity tests. Interestingly, the susceptibility results confirmed that the transformed *E. coli* S1 cells have become resistant to Ceftazidime and Penicillin G (Figure 7). Given that the ccompetent *E. coli* HST08 strain represents sensitive modified bacteria, it's obviously the transparent area of the inhibition zone would appear around the tested antibiotics, except the

cells that become transformed with *P. aeruginosa* plasmid DNA that harbors β-lactam genes which encode for Ceftazidime and Penicillin G resistance (23,14). To sum up with, the findings clearly indicate the key role of plasmid DNA in augmentation of *P. aeruginosa* horizontal resistance because the antimicrobial agents are known to belong to β-lactam class of antibiotics (21,22).

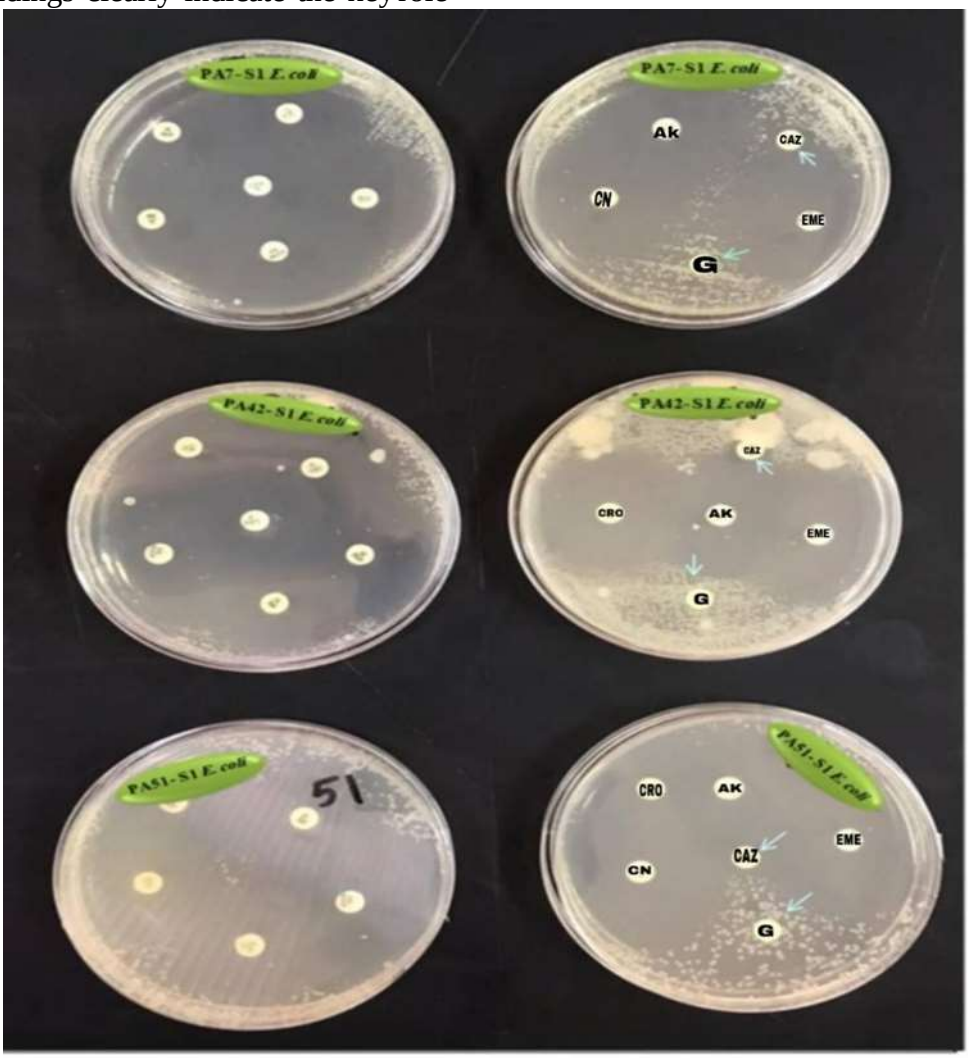

Figure 7. The figure displays the antibiotic susceptibility testing results of the transformed *E.coli* S1

It worth to state that the inhibition zones demonstrated in the figure 3-11 appears irregular due to antibiotic synergy due to the presence of synergistic antibiotics (AK, EME, CN) with Ceftazidime. This may indicate that the produced cephalosporinase are ineffective against this synergistic action. In addition to that the competent cells are originally hypersensitive to antibiotics. This could be the possible explanation for appearing the overlapped inhibition zones (15).

CONCLUSION

The results of bacterial screening confirm the previous reports of domination of *P. aeruginosa* almost in respiratory tract infections. Most of the *P. aeruginosa* isolates were resistant to the antibiotics "ceftazidime and ceftriaxone". The examined *P. aeruginosa* isolates harbour low molecular weight of plasmids, which confer resistance to beta lactam antibiotics "ceftazidime and penicillin G". The results indicate that bacterial HGT has a key role in emerging antibiotics resistance in *P. aeruginosa* causing respiratory infections.

ACKNOWLEDGMENT Sincerely thank to the biologist Asmaa Alkabtan/Tuberculosis Institute / The Medical City, the staff of Al-Muhmodia Public Hospital / Bacteriology lab and Dr.- Intesar Al Ramahi Director of Ibn Sina Center/ Ministry of Industry and Minerals for their continuous support and for providing all required facilities to perform this research.

REFERENCES

1.Arai, H. 2011. Regulation and function of versatile aerobic and anaerobic respiratory metabolism in *Pseudomonas aeruginosa*. Front Microbiol 2, 103.

2.Berube, B. J. S. M., Rangel and A. R.Hauser. 2016. *Pseudomonas aeruginosa*: breaking down barriers. Curr Genet 62, 109-113.

3.Borman, A. M. J., Muller; J., Walsh-Quantick; A., Szekely; Z., Patterson;M. D., Palmer and E. M. Johnson. 2020. MIC distributions for amphotericin B, fluconazole, itraconazole,voriconazole, flucytosine and anidulafungin and 35 uncommon pathogenic yeast species from the UK determined using the CLSI broth microdilution method. Journal of AntimicrobialChemotherapy, 75(5), 1194-1205.

4.Carattoli, A.; E., Zankari; A., García-Fernández; M., Voldby Larsen; O., Lund; L., Villa and H. Hasman. 2014. In silico detection and typing of plasmids using plasmid finder and plasmid multilocus sequence typing. Antimicrobial agents and chemotherapy, 58(7), 3895-3903.

5.Carmeli, Y., J., Armstrong; P. J., Laud; P., Newell G.,; Stone; A., Wardman and L. B. Gasink. 2016. Ceftazidime-avibactam or best available therapy in patients with ceftazidime-resistant Enterobacteriaceae and *Pseudomonas aeruginosa* complicated urinary tract infections orcomplicatedintra- abdominal infections (REPRISE): a randomized pathogen-directed, phase 3 study. The Lancet Infectious Diseases 16, 661-673.

6.Carroll, I. M. T., Ringel-Kulka; J. P., Siddle; T. R., Klaenhammer and Y. Ringel. 2012. Characterization of the fecal microbiota using high- throughput sequencing reveals a stable microbial community duringstorage. PloS one, 7(10), e46953.

7.Chun, L. Y. L., Dolle-Molle; C., Bethel; R. C., Dimitroyannis; B. L., Williams; S. A., Schechet and D. Skondra. 2019. Rapid pathogen identification and antimicrobial susceptibility testing in in vitro endophthalmitis with matrix assisted laser desorption-ionization Time-of- Flight Mass Spectrometry and VITEK 2 without prior culture. Plos one, 14(12), e0227071.

8.Crousilles, A.; E., Maunders; S., Bartlett; C., Fan; E. F., Ukor; Y., Abdelhamid and M., Welch.2015. Which microbial factors really are important in *Pseudomonas aeruginosa* infectios?. Future microbiology, 10(11), 1825-1836.

9.Deshpande, R. and C. Zou, 2020 *Pseudomonas aeruginosa* Inducedcell death in acute lung injury and acute respiratory distress syndrome. Int J Mol Sci 21.

10.Don, J.; R. Noel and T. James. 2005. Bergey's Manual-of Systematic Bacteriology. (2PndP ed.). contributions from 339 colleagues.

11.Dubnau, D. and M. Blokesch. 2019. Mechanisms of DNA uptake by naturally competent bacteria. Annu Rev Genet 53, 217-237.

12.El Chakhtoura, N. G. E., Saade; A., Iovleva; M., Yasmin; B., Wilson; F., Perez and R. A. Bonomo.2018. Therapies for multidrug resistant and extensively drug-resistant non-fermenting gram-negative bacteria causing nosocomial infections: a perilous journey toward 'molecularly targeted' therapy. Expert Rev Anti Infect Ther 16, 89-110.

13.Eyns, H. D., Pierard; E., De Wachter; L., Eeckhout; P., Vaes and A. Malfroot. 2018. Respiratory bacterial culture sampling in expectorating and non-expectorating patients with cystic fibrosis. Front Pediatr 6, 403

14.Fadhel Abb, S., A. S. K., Al-Khafaji and H. M. Radif. 2018. Investigation of plasmid-associated fluoroquinolone resistance in nosocomial *Pseudomonas aeruginosa* isolated from infected burn wounds. Journal of Biological Sciences 18, 514-519.

15.Faqri, A. A. N. H., Hayder and A. J. Hashim. 2019. Lab-scale production of rhamnolipid by a3 and study its synergistic effect with certain antibiotics against some pathogenic bacteria. Iraqi Journal of Agricultural Science, *50*(4), 1290- 1301. https://doi.org/10.36103/ijas.v50i5.794

16.Galetti, R., L. N., Andrade; M., Chandler; M., Varani Ade and A. L. Darini.2016. New small plasmid harboring blaKPC-2 in *Pseudomonas aeruginosa*. antimicrob agents chemother 60, 3211-3214.

17.Gupta, R. and M. Schuster. 2012. Quorum sensing modulates colony morphology through alkyl quinolones in *Pseudomonas aeruginosa*. BMCmicrobiology, 12(1), 1-12.

18.Higgins, N. P. and A. V. Vologodskii. 2015. Topological behavior of plasmid DNA. Microbiol

Spectr 3.
19.Hoiby, N., O., Ciofu and T. Bjarnsholt. 2010. *Pseudomonas aeruginosa* biofilms in cystic fibrosis. Future microbiology, *5*(11), 1663- 1674.
20.Ibrahim, A. H. 2022. Link between some virulence factors genes and antibacterial resistance of Pseudomonas aeruginosa. Iraqi Journal of Agricultural Sciences, 53(5), 985-993. https://doi.org/10.36103/ijas.v53i5.1612
21.Hussein, N. N. and A. H. Muslim.2019. Detection of the antibacterial activity of AgNPs biosynthesized by *Pseudomonas aeruginosa*. Iraqi Journal of Agricultural Science, 50(2), 617-625. https://doi.org/10.36103/ijas.v2i50.661
22.Li, H. M. A., Toleman; P. M., Bennett; R. N., Jones and T. R. Walsh.2008. Complete sequence of p07-406, a 24,179-base-pair plasmid harboring the blaVIM-7 metallo- beta-lactamase gene in *a Pseudomonas aeruginosa* isolate from the United States. Antimicrob Agents Chemother 52, 3099-3105.
23.Manjunath, S. V. R. S., Baghel and M. Kumar 2020. Antagonistic and synergistic analysis of antibiotic adsorption on Prosopis juliflora activated carbon in multicomponent systems. Chemical Engineering Journal, 381, 122713.
24.Nafee, S. K. 2012. Isolation And Identification of Clinical *Pseudomonas aeruginosa* Producing Exotoxin A And Studying Its Toxic Effect In Mice (Doctoral dissertation, thesis. MV Sc. College of Science/Baghdad Univ. Master of Science in Biotechnology).
25.Nolan, L. M.; L., Turnbull; M., Katrib; S. R., Osvath; D., Losa; J. J., Lazenby and C. B. Whitchurch. 2020.*Pseudomonas aeruginosa* is capable of natural transformation in biofilms. Microbiology (Reading) 166, 995- 1003.
26.O'Neill, J. 2016. Tackling drug- resistant infections globally: final report and recommendations.
27.Qasim, D. A. 2019. Molecular detection of Pseudomonas aeruginosa isolated from minced meat and studies the pyocyanin effectiveness on pathogenic bacteria. Iraqi Journal of Agricultural Sciences, 50(4):1199-1207. https://doi.org/10.36103/ijas.v50i4.764
28.Qureshi, H.; A., Sharafkhaneh and N.Hanania. 2014. Chronic obstructive pulmonary disease exacerbations: latest evidence and clinical implications. Therapeutic advances in chronic disease, 5(5), 212-227.
29.Siddalingappa, C. M.; L., Kalpana; S., Puli; T. K., Vasudha and A. Acharya. 2013. Sensitivity pattern of bacteria causing respiratory tract infections in a tertiary care centre. International Journal of Basic and Clinical Pharmacology, 2(5), 590- 595.
30.Thomas, M. and P. A. Bomar. 2020. Upper respiratory tract infection. StatPearls.
31.Tawfiq, S. M. 2018. Bacteriologigal and genetic study of *Pseudomonas aeruginosa* isolates. Iraqi Journal of Agricultural Sciences, 49(1): 27-35. https://doi.org/10.36103/ijas.v49i1.201
32.Wang, Y. J., Wang; R., Wang and Y. Cai. 2020. Resistance to ceftazidime-avibactam and underlying mechanisms. J Glob Antimicrob Resist 22, 18-27.