Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Effectiveness of Green Synthesized ZnO Nanoparticles Against MDR Resistant Klebsiella Pneumoniae Isolated From Diabetic Foot on Some Virulence Factors Genes Expression

HaiderTurky Mousa Al-Mousawi[1], Mustafa Muhammed Jadooa[2], Nadhim Mushtaq Al-bderee[3]
[1,2,3] Department of Applied Biotechnolog, College of Biotechnolog, Al-Qasim Green Universit, Babylon 51013, Iraq
ORCID ID: https://orcid.org/0000-0001-9292-4611

Email: dr.haider almusawi@biotech.uoqasim.edu.iq

Abstract. Diabetic foot ulcers are a major public health problem and are quite costly for diabetic patients. Besides, patients with diabetic foot ulcers often have a poor quality of life. Most of the main complications of diabetic foot ulcers are the bacterial infection that leads to gangrene when different types of antibiotics fail to prevent bacterial infections. The research proposed in this study aims to determine the most effective synthesis type of Zinc oxide nanoparticles (ZnO NPs) and study its effect on some virulence factor genes expressions. Research includes the isolation and identification of several bacterial species by biochemical tests, as well as confirmation by the Vitek system and molecular diagnostics. Out of 100 swabs, 26 samples were found to be contaminated with bacteria Klebsiella pneumoniae is one the main virulence factor that develops of diabetic foot ulcers. The biological production of Zinc Oxide was using bacteria Pseudomonas aeruginosa. The characterization of the Zinc Oxide products was using the Ultra-violet Visible spectrophotometer, Fourier transform infrared spectroscopy, Scanning electron microscopy, X-Ray diffraction spectrum very small crystals with a large spherical shape were formed, with a size of 13.5 nm. The genes were included as the bacterial virulence factors coding genes, MagA of gene Klebsiella pneumoniae, which are responsible for the production of exotoxins that cause sepsis, necrosis, tissue necrosis and non-healing. The expression of MagA genes the different before and after treatment with ZnO NPs. The mRNA for both genes was extracted alongside the mRNA for 16SrRNA acting as housekeeping gene. All mRNAs were converted to cDNA through RT-PCR technique and then measure the expression by qPCR. The expressions of MagA genes for the biological product nanomaterial, bacterial nanomaterial, were significant for all the isolates with $P<0.01$. In conclusion from the foregoing the biological nanoparticles product was more sufficient for inhibition of most isolates.

Highlights:

1. Diabetic foot ulcers worsen by bacterial infections, causing severe complications.
2. ZnO nanoparticles synthesized biologically; bacterial virulence gene expression analyzed.
3. ZnO nanoparticles significantly inhibited K. pneumoniae virulence with $P<0.01$.

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Keywords: K. pneumoniae, P. aeruginosa, ZnO NPs, Mucoviscosity associated a gene (MagA) and gene expression

Introduction

Diabetic Mellitus (DM) is characterized by a rise in blood glucose levels known as hyperglycemia that is caused by an autoimmune response that damages pancreatic cells, resulting in insulin shortage, carbohydrate metabolism and the resulting insulin effect are the basis of the pathophysiology of diabetes mellitus 1. Diabetes mellitus has been increasingly predominant over the latest decades, where the expectation of DM occurrence is increased to 592 million globally by 20352. It was found that 15% of patients with this disease are exposed to diabetic foot ulcers3. The β cells in langerhans pancreas organ controls the levels of blood glucose and whose destruction is termed as diabetes mellitus this physiological function of glucose-cell contact for energy production is disrupted or perhaps entirely impaired if pancreatic cells do not secrete and glucose levels in the blood do not decrease but instead rise, resulting in hyperglycemia, Another cause of hyperglycemia is when target cells fail to use the pancreatic cell or insulin, TIDM and T2DM , two types of diabetic: TIDM is an insulin-dependent form of diabetes in which autoimmune cells destroy insulin-producing cells, resulting in a reliance on insulin for survival, the most frequent (T2DM) which is causes with insulin resistance with the tissues as well as increasing insulin resistance4. DFUs are one of the many problems brought on by uncontrolled diabetic, and are considered a serious condition of diabetes is foot and lower leg ulcers5. The antibiotic resistance and overuse of antibiotics which has resulted because of their increased effectiveness against resistant bacteria, lower toxicity, and heat resistance, different NPs can be used as antimicrobials to prevent emergency and propagation of pathogens and resistant genes in harmful bacteria, between many metal oxides, ZnO has a number of noteworthy qualities, including biological and chemical stabilisation, high photocatalyst, and good antimicrobial function6. Zinc Oxide NPs therapeutic agent for diabetic ulcers infections and its associated wound healing, this is made possible as the zinc element is associated with normal wound healing and its function in more than 300 enzymes7. Nanomaterials remove contaminants in a number of ways, including attaching to the internal proteins, inactivated them, producing ROS, and directly damaging bacterial cell walls, as was the situation of Nanoparticles NPs' antimicrobial properties6.

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Methods

The Samples Collections

One hundred swab of DFUs was collected from diabetic mellitus admitters in (General Al-Hashmia Hospital) during November 2021 to February 2022, the patients have clinical features swelling, redness, skin discoloration, skin sores, numbness, pain, tingling, foul smell, wounds and discharge of fluid or pus.

Samples Collection

The swab was collected under sterile conditions from patients infected with DFU after removing pus and unwanted materials and before using sterile materials and solutions used for lavage during a period of 4 months from November to February 2022. The sample was transferred directly to the laboratory for cultivation, isolation and diagnosis of pathogenic bacteria.

Prepare and Sterilization of culture media

All ready culture media agars, broths and indicators were prepared according for manufacturing company instructions; the constituents were dissolved in D.W, for dissolve the all constituents completely, sterilized by using autoclaving at item 121ºC - 15min. After cooled to 45-50°C, each medium was dispensed into sterile petri dishes or test tubes as required, media stored in a refrigerator 4-8˚C until use to ensure their safety8.

Identification of Isolates Based on 16SrRNA Sequence

A 16SrRNA primer was used mainly to identify this same isolates. RNA is an evolutionary marker that really is available throughout all pathogens and now has got to play a significantly roles in evolution of microorganisms phylogenetic, classification. Bacterium are already characterized by the common ancestry of their sequenced genomes, so any crude extract could be distinguished with great certainty if the nucleotide sequences of said type varieties indicating all the famous bacterial species were available9.

Synthesized of the ZnO – NPs

Biosynthesis ZnO NPs by Pseudomonas aeruginosa extract

Collection of Pseudomonas aeruginosa

The soil isolated bacterial was obtained P. aeruginosa by at the University of Babylon / College of Science for women, which was cultivated again.

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Identification of P. aeruginosa.

The isolated bacteria were identified depending on morphological diagnostic, microscopic characterization, biochemical tests, Vitek-2 system for activation and do all the standard diagnostic tests as well to ensure the isolate10.

Preparation of Bacterial Suspension

The bacterial isolate used for research was selected by taking a number of single colonies of the selected isolate (bacterial growth) and inoculated in 250 ml of sterile brain heart medium for 24 h at 35±2°C in a 500 mL Erlenmeyer flask to grow, under shaking conditions at 120 rpm on an orbital shaking incubator, then the sample was filtered after centrifugation using filter paper with a diameter of 0.45 micrometer, then the filter was taken for use in preparing the preparation of ZnO NPs11.

Synthesis of Zinc Oxide Nanoparticles

One hundred ml of the bacterial filtrated were taken, was mixed with 100 mL of solution (1mM) Zinc acetate Zn(CH3COO)2.H2O2in a 500mL Erlenmeyer flask, incubated for 96 hours at 32°C with agitation at 150 rpm, ZnO NPs were obtained by centrifuging at 1000 rpm for 10 minutes, after which the precipitant washed once with 96% ethanol and then dried in an oven at 60°C, and weight of ZnO NPs10;12.

Method (Agar –Well- Diffusion)

The antibacterial activity was evaluated used different concentrations of biosynthesis and chemical synthesis ZnO NPs on all bacterial isolate that collected from patients DFUs, by agar well diffusion method13. According to14, the test done on Muller Hinton agar with different concentrations of (ZnO - NPs).

Result and Discussion

The isolated microorganisms from diabetic foot ulcers sources, which were the identified using the Microscopic, Macroscopic, the Biochemical tests, results confirmed by the ViteK-2 System, and molecular identification, revealed that the most frequently isolated bacteria, which was consistent with previous findings15. The identity was then verified using the Biomerieux-recommended VITEK 2 compact equipment, and the resulting data was compared with the source supplied by16,17

The DNA Extraction

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

For this study, the identified of pathogenic bacteria clinical isolated through DNA of bacterial isolates, were isolates seven K. pneumoniae. Also the purity, concentration were confirmed at was Nano-drop, the nucleic acid is purity from samples at range between (1.8-2). Purity of DNA is a good indicator of the extraction process as well as confirming the absence of impurities that could impede the process15. Molecular identification the (genomic-DNA) was successfully extracted of isolated bacteria in this study through a commercial the (genomic-DNA) purification kit (FAVORGEN) according to instructions of the company, extraction results were good for DNA, the extraction DNA was confirmed, analytic by the horizontal gel electrophoresis in (1)% agarose for 45 min at 100 volts and was exposed to UV light where the DNA appears as compact bands18. As shown in Figure (1).

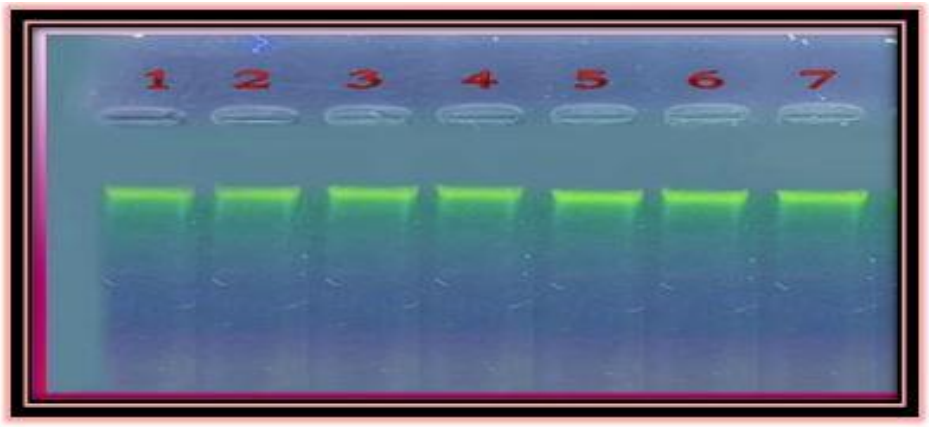

Figure (1): Presented the integrity of DNA which extracted from pathogenic bacteria isolated from DFUs., K. pneumoniae (1 7___) ; Agarose 1.3%, Volt 100, Time 45 minutes.

Identification of Bacterial Isolate by Molecular Detection (Universal 16SrRNA gene)

The isolates, and the gene of interest was PCR amplified using universal bacterial primers to identify the 16SrRNA gene of all isolates, is the Polymerase Chain Rraction and this produced were tested in electrophoresis, the agarose gel analysis and photographed under UV light, showed clear pure bands, compared with the DNA ladder, PCR product size (1239) bp was corresponding to the standard DNA ladder size of 1500 bp as presented in Figure (2). The result of the current study agree with19,20 identified the following bacterial isolates: K.pneumoniae. The sequence analysis was performed

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

using the (BLAST) of the (NCBI) to the 16SrRNA gene sequence for these isolates that aligned, sequencing 16sRNA gene sequencing for bacterial isolates were received at online, aligned to the NCBI data base using the blast software and aligned is multiple for each other using the Bio-Edit software, the submitted in the fasta format to NCBI through sequin software, that sequencing of the isolated explain similar with (NCBI) information, above results were consistent with this results of biochemical, morphological identification for these isolates.

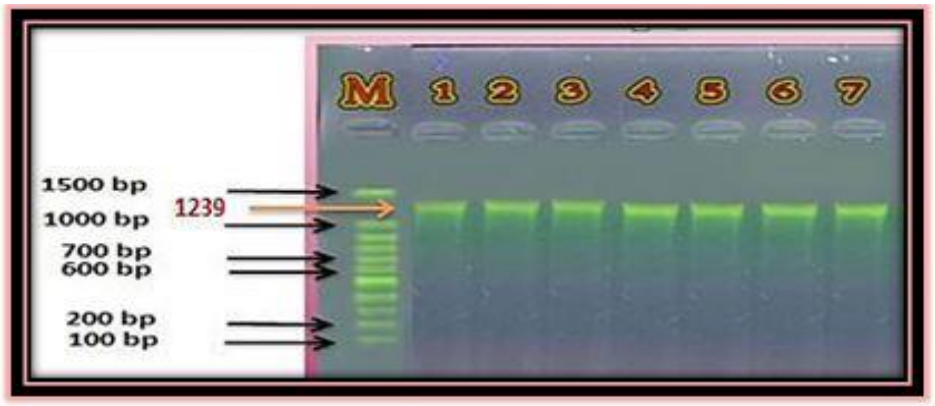

Figure (2): Presented Amplification of *16SrRNA* to molecular conform DNA of pathogenic bacterial which isolated from DFUs., M:100 bp DNA *K. pneumoniae* (1 —7). Agarose 1.3%, Volt 100, Time 45minutes.

Production of The Zinc - Oxide NPs

Biosynthesis of ZnO NPs by using Bacteria

Identification of P. aeruginosa

The characteristics results of the bacteria on the MacConkey medium were pale in color due to their inability to ferment lactose, on the blood agar that showed transparent halo evidence of hemolysis and production of hemolysin. While on the nutrient agar they appeared with color green, microscopically they were Gram negative bacilli as well as the shape and arrangement of cells and the biochemical examinations results of P.aeruginosa showed that isolate had given negative result for indole, urease test. While citrate was positive, oxidase positive and TSI alkaline/alkaline with no production of H2S and gas. These results agreed with what mentioned by21. Similar finding were recorded by22.

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Biosynthesis of ZnO NPs by using Pseudomonas aeruginosa

The results of the current study showed the appearance of a precipitate at the end of the reaction process, and this is evidence of the biosynthesis of ZnO NPs, The ZnO NPs were obtained white powder after drying, their weight was taken. The filtrate extract of these bacteria P.aeruginosa was used as a reducing agent and stabilizer for the purpose of biosynthesis of ZnO NPs, an way safe and economical, low toxicity, as indicated23.

They got a product ZnO NPs, mediated by extracellular secondary metabolite produced by P.aeruginosa, which stated that microbial enzymes play an effective and essential direct role in the biosynthesis of ZnO NPs, pick up the target ions from the surrounding environment and then reduced of the metal ions into elements through their enzymes that release through the metabolic and cellular activity.

Characterization of (ZnO - NPs)

The UV–Visible Spectrophotometer

A highest the absorption peaks for ZnO-NPs was observe at 390 nm as shown in Figure (3)., this result indicates the successful biosynthesis of ZnO - NPs by bacterial filtrate. The successful biosynthesis of the ZnO - NPs by a confirmed by the color changing to the white precipitate The results of this-study are in agreement with the highest absorption was observed when biosynthesizing zinc particles from P.aeruginosa was 390 nm. It was identical to the result of24.

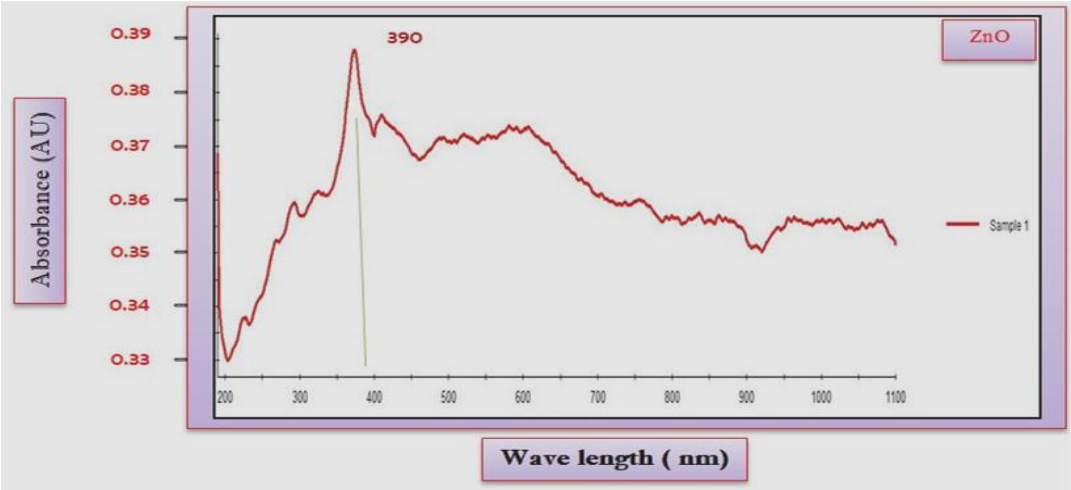

Figure (3): Show UV of biosynthesis ZnO NPs

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

X-ray Diffraction Characterization

The XRD pattern showed to bacterial biosynthesis intense diffraction peaks at 2 values of respectively, this results which confirmed using the material (Zinc Oxide) that is high purity, When compared to X-ray diffraction, our observations were consistent with the crystalline character of biologically generated ZnO-NPs, and the presence from bacterial secondary metabolites including: (proteins and chemical compounds) that coated the (ZnO – NPs) surface was strongly indicated 24, 25. The average of the ZnO NP size that matched the highest diffraction peaks was determined to be 13.5 nm, as presented in Figure (4).

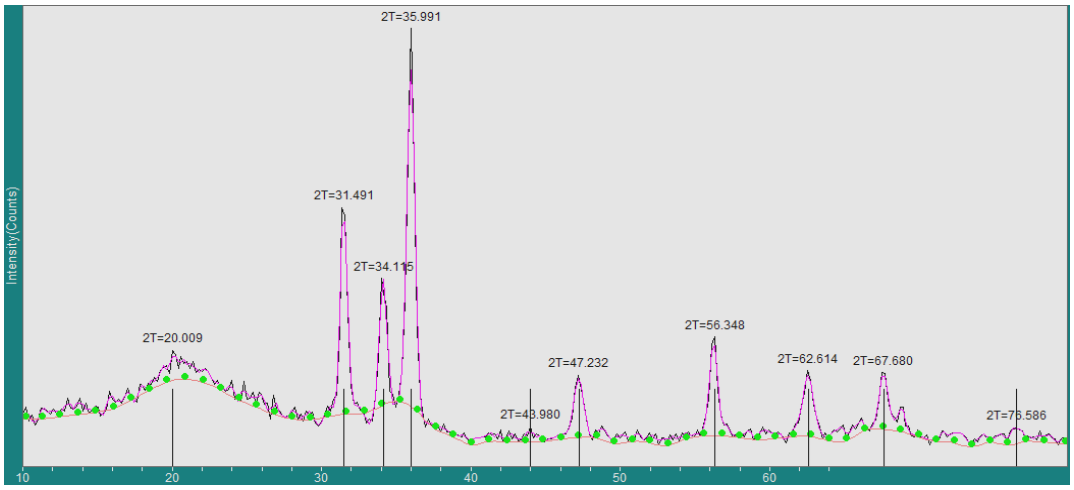

Figure (4): XRD graph of biosynthesis ZnO NPs

Scanning Electron Microscope (SEM)

The results showed the phenotypic features of ZnO NPs by the bacterial filtrate, regular structures spherical. In addition to the particle size as show in Figure (5). Our study succeeded in achieving good results with the smallest sizes of ZnO NPs, which were less than what was found 26. Also 27, indicated in his research that the average size ranged between (50-120) nm, and [28], he indicated his results for ZnO NPs biosynthesized by with the average Size between (20-40) nm. The current study succeeded in achieving the best and most accurate results at the level of the small sizes of ZnO NPs, and the originality of good results for biosynthesis.

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

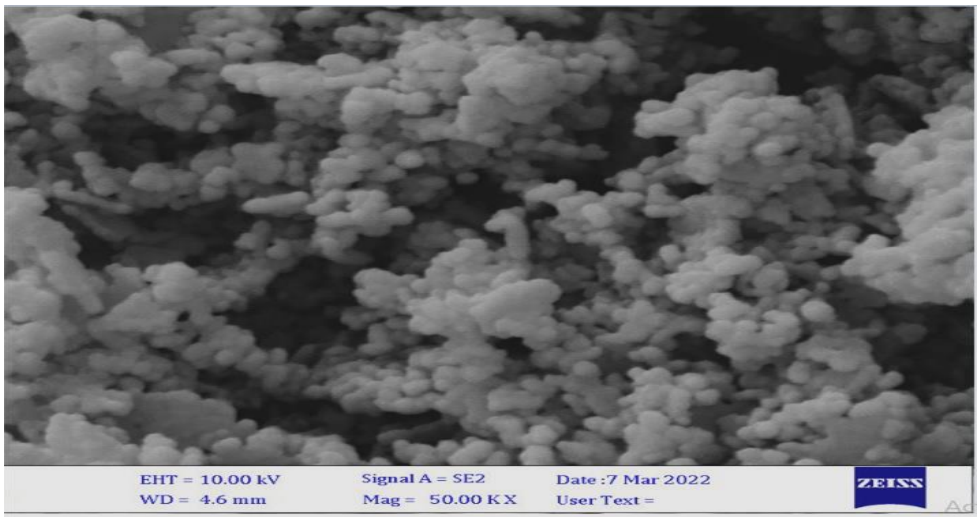

Figure (5): SEM image of biosynthesis ZnO NPs

Fourier Transformed Infrared (FTIR)

The synthetic (ZnO - NPs) through used the bacterial was discovered using FTIR as presented in Figure (6). The stretching NH of amines was represented by the overlap of vibration of (-OH) group by the wave number, whereas the stretching (O-H) for carboxylic acid was represented by peaks at wave numbers between 3000 and 3700 cm-1 28. While the faint signal the main amine (NH), which was bending, overlapped with the carboxylated salts or amide, and the stretching thiol group (SH) was given the peaks at (2590) cm-1. 29. Stretching of carboxylic salts' C=O during CO2 and carbonates (CO32) adsorption at the NPs surface was also shown by the medium peaks (1430) cm-1. 30. Sulfoxide stretching (S=O) and alkene bending (C=C) may be responsible for the strong peaks at (1032) cm-1, (895) cm-1, respectively. 30; 31. Peaks at (400 - 700) cm-1 confirmed the generation of ZnO NPs, which was previously reported32. The (O-H), (C=O), (NH), and (S-H) groups, which are present of bacterial that reducing, the capping, and the stabilizing functions, are among the groups implicated, was validated by the FTIR analysis of ZnO-NPs.

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

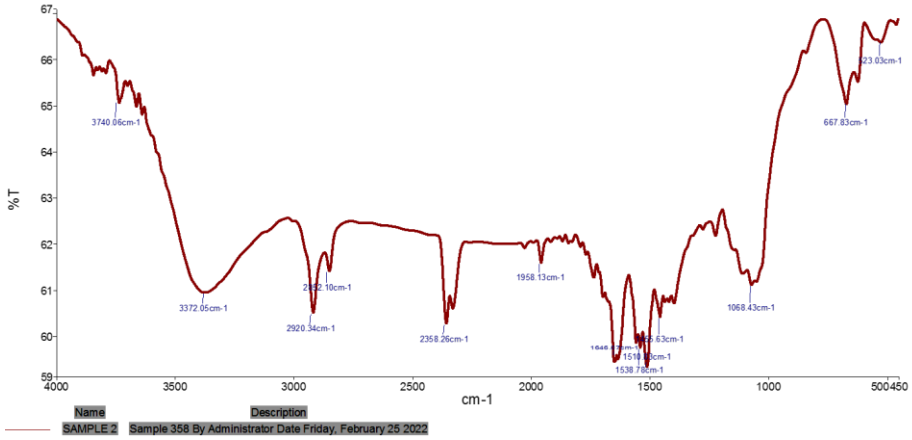

Figure (6): FTIR of biosynthesis ZnO NPs

Agar Well Diffusion Method

The results showed of ZnO NPs bacterial product by P.aeruginosa give that the bacterial product was recorded at K. pneumoniae a concentration of 500 µg/ml inhibition zone diameter (16.8) mm, while it was recorded at a concentration of 250 µg/ml (11.0) mm, as well as the concentration of 125 µg/ml recorded as an inhibition zone with a drop (6.6) mm, and the other concentrations were unable to prevent bacterial growth.

Pathogenic bacterial	No. of Isolates	ZnO NPs by *P. aeruginosa*					P-value
		Concentrations (µg/ml)					
		31.25	62.5	MIC 125	250	500	
K. pneumoniae	K1	Non	Non	5.4	10.2	15.0	0.0001 **'
	K2	Non	Non	6.4	11.2	16.0	0.0001 **'
	K3	Non	Non	6.6	10.6	16.8	0.0001 **'
	K4	Non	Non	6.0	11.0	16.0	0.0001 **'
	K5	Non	Non	6.0	11.4	16.3	0.0001 **'
	K6	Non	Non	6.4	11.0	16.6	0.0001 **'
	K7	Non	Non	6.3	10.2	14.4	0.0001 **'

Real Time qPCR of 16SrRNA Gene Expression

In the present study, the internal control gene 16SrRNA was used, the range of Ct values for this gene when not treated with ZnO NPs was from (14.55 - 14.68) in all (7) K. pneumoniae isolates from DFUs, the isolates treated with synthesized ZnO NPs, the Ct value did not change at a high rang from (14.03-14.99), as showed in Table (2),

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

the statistical analysis showed a non-significant difference between isolates before and after treatment. Figures (7) showing the pattern of gene amplification.

Table (2): The Ct Value of (Housekeeping16SrRNA gene) before

No. of isolates	The Ct value of *(HK)* gene before treatment	The Ct value of *(HK)* gene after treatment
HK-1	14.92	14.92
HK-2	14.88	14.87
HK-3	14.81	14.03
HK-4	14.77	14.25
HK-5	14.80	14.67
HK-6	14.73	14.54
HK-7	14.71	14.22
NS: Non-Significant		

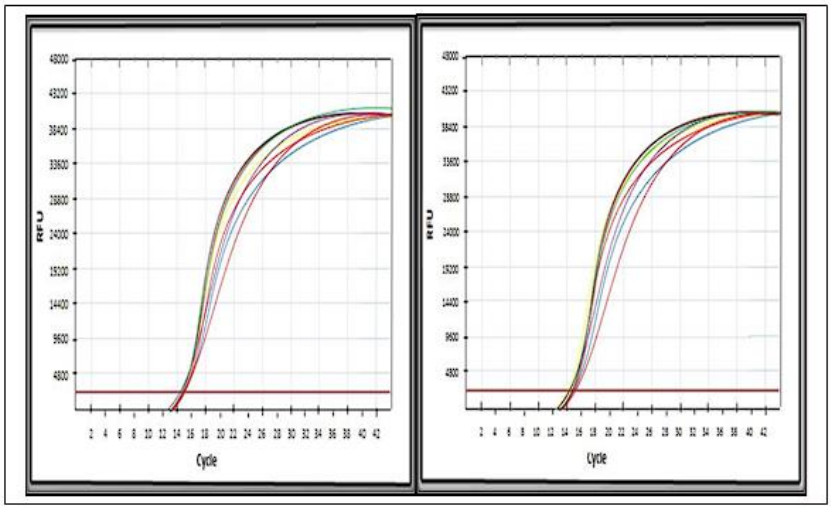

Figure (7): The Amplification of 16SrRNA Gene before and after Treatment with ZnO NPs.

Real Time qPCR of MagA Gene Expression

Demonstrated q RT-PCR using seven isolates of K. pneumoniae from diabetic foot ulcer patients before and after treatment with ZnO NPs, the results of the MagA gene,

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

which gave high expression for all isolates and with different degrees of the bacterial biosynthesis product ZnO NPs. The treatment with the bacterial biosynthesis product ZnO NPs, as it gave a high expression with an increase in Ct value and it was statistically $P<0.01$ evidence of occupational differences that were between the isolates before and after treatment as in Table (2) and Figures (8) showing the pattern of gene amplification. Their differences depend on the range of the Ct values as untreated and treated. By comparing the results on the extent of the Ct value of the gene when treating bacterial isolates with ZnO NPs. As a result of the biological product's possession of biologically active functional groups such as the amine group, the carboxyl group and others and merely touching the surface of the cell, the cell generates electrical charges that damage its membranes and disrupts the genes of the cell membranes, and its influence stops cellular activities and thus the death of the cell. As indicated33,34.

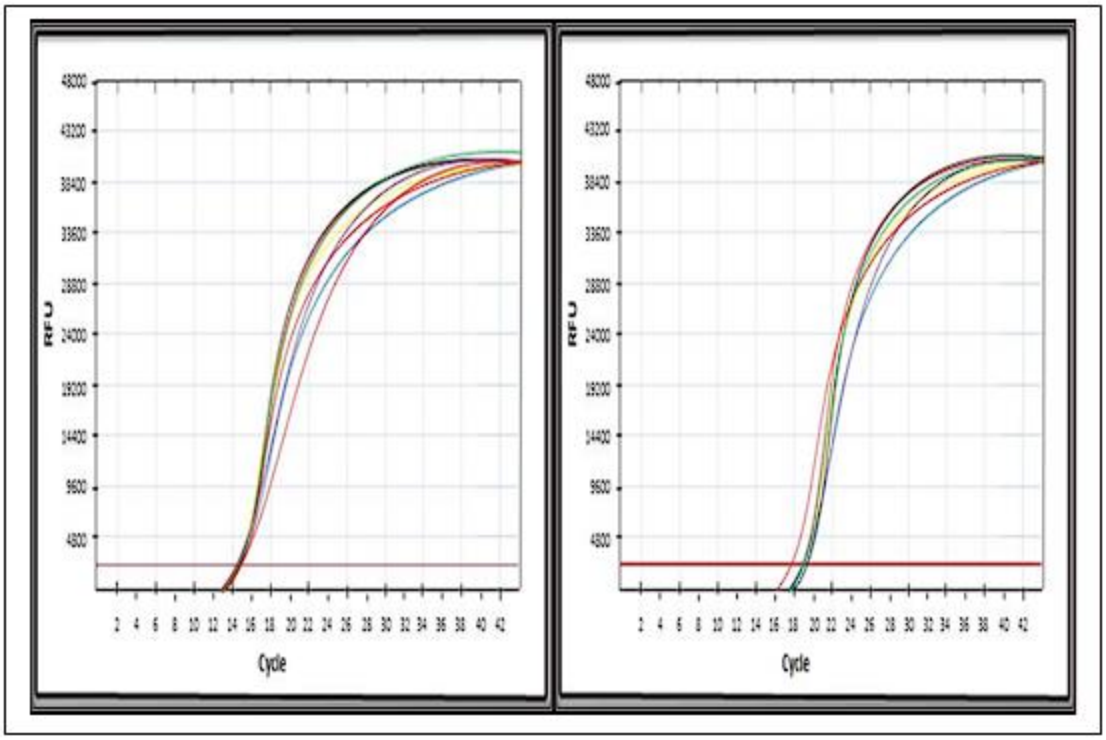

Figure (8): The Amplification of MagA gene before and after treatment with ZnO NPs.

Indonesian Journal on Health Science and Medicine
Vol. 2 No. 1 (2025): January
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Table (2): Gene Expression of MagA gene (Before and After Treated with ZnO NPs)

No. of isolates	Treatment ZnO NPs *A.niger*	Ct *(MagA)* of Target gene	Ct of reference *(HK) gene*	ΔCt Target (Ct *(MagA)* - Ct *(HK)*	ΔΔCt (ΔCt – Ave ΔCt)	$2^{-\Delta\Delta Ct}$ Fold of gene expression	P-value
K1	Untreated	14.81	14.92	-0.11	-0.548	1.46 ±0.24	0.0063 **
	Treated	18.66	14.92	3.74	3.30	0.10 ±0.02	
K2	Untreated	14.87	14.88	-0.01	-0.448	1.36 ±0.12	0.0067 **
	Treated	18.99	14.87	4.12	3.68	0.077 ±0.08	
K3	Untreated	14.86	14.81	0.05	-0.388	1.30 ±0.14	0.0058 **
	Treated	19.04	14.03	5.01	4.57	0.042 ±0.005	
K4	Untreated	14.72	14.77	-0.05	-0.488	1.40 ±0.12	0.0053 **
	Treated	17.77	14.25	3.52	3.08	0.118 ±0.07	
K5	Untreated	14.70	14.80	-0.1	-0.538	1.45 ±0.13	0.0050 **
	Treated	19.28	14.67	4.61	4.17	0.055 ±0.008	
K6	Untreated	14.74	14.73	0.01	-0.428	1.34 ±0.14	0.0075 **
	Treated	18.63	14.54	4.09	3.65	0.079 ±0.004	
K7	Untreated	14.69	14.71	-0.02	-0.458	1.37 ±0.16	0.0087 **
	Treated	18.75	14.22	4.53	4.09	0.058 ±0.007	

** (P≤0.01).

q-Real Time-Polymerase Chain Reaction Evaluation

By comparing the Cycle threshold in (RT-PCR) of the target gene Cycle threshold (MagA) to internal control Cycle threshold (16S), it was possible to determine the relative expression of the hla gene: Ct MagA-Ct 16S is equals to the delta Ct. the relative gene expression to the three biological replicated was averaged to assess the experiment's repeatability. The standard deviation of the Delta Ct obtained to each the biological replica was calculated to assess the RT-PCR reaction's repeatability35,36,37

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Conclusion

Efficiency of ZnO to inhibit on isolated bacteria, which had the potential for down regulate MagA gene expression in this study. Our study succeeded where it was distinguished with biosynthesis ZnO NPs, the study that have smaller size when compared with previous studies, which is preferable to the achieve better anti-microbial effects. As a result of the biological products possession of biologically active functional groups such as the amine group, the carboxyl group, and others, merely touching the surface of the cell, generates electrical charges that damage its membranes and disrupts the genes of the cell membranes, and its influence stops cellular activities and the death of the cell as indicated. The synthesis of hydrogen peroxide and eventual penetration of cell envelope and disorganization of bacterial membrane following contact with ZnO NPs are the two potential mechanisms linked to bacterial inhibition

References

[1] M. Gadelkarim, A. I. Abushouk, E. Ghanem, A. M. Hamaad, A. M. Saad, and M. M. Abdel-Daim, "Adipose-derived stem cells: Effectiveness and advances in delivery in diabetic wound healing," Biomedicine and Pharmacotherapy, vol. 107, pp. 625–633, 2018.

[2] T. C. Quinn, D. A. Piggott, K. M. Erlandson, K. E. Yarasheki, D. Vancampfort, J. Mugisha, and B. Stubbs, "Global estimates of diabetes prevalence for 2013 and projections for 2035," J. Clin. Exercise Physiol., vol. 8, no. 2, pp. 86–90, 2019.

[3] P. G. Roy, K. Kumar, N. P. Singh, G. S. Ranga, and S. Giri, "Microbiological profile and antibiotic sensitivity pattern of bacteria in diabetic foot ulcers in a tertiary care hospital," J. Adv. Res. Med., vol. 8, no. 1, pp. 2394–7047, 2021.

[4] S. Wahjuni and N. W. Bogoriani, "Green Mustard Ethanol Extract (Brassica Rapa L.) Leaf Can Cell Damage (8-Hydroxy-2-Dioxiguanosine) in the Wistar Rat Hyperglycemic," in IOP Conf. Ser.: Earth Environ. Sci., vol. 709, no. 1, 2021, p. 012046.

[5] X. Li, H. Kou, C. Zhao, F. Zhu, Y. Yang, and Y. Lu, "Efficacy and safety of ALA-PDT in treatment of diabetic foot ulcer with infection," Photodiagnosis and Photodynamic Therapy, vol. 38, p. 102822, 2022.

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

[6] R. Ishwarya, B. Vaseeharan, S. Kalyani, B. Banumathi, M. Govindarajan, N. S. Alharbi, and G. Benelli, "Facile green synthesis of zinc oxide nanoparticles using Ulva lactuca seaweed extract and evaluation of their photocatalytic, antibiofilm, and insecticidal activity," J. Photochem. Photobiol. B: Biol., vol. 178, pp. 249–258, 2018.

[7] R. V. Kumar, S. Vinoth, V. Baskar, M. Arun, and P. Gurusaravanan, "Synthesis of zinc oxide nanoparticles mediated by Dictyota dichotoma endophytic fungi and its photocatalytic degradation of fast green dye and antibacterial applications," South Afr. J. Bot., vol. 151, pp. 337–344, 2022.

[8] E. C. Constance, C. Perpetua, and S. A. Steve, "Media and Air Sterilization Required for Microbial Fermentation," Constance Chinyere Ezemba, 2020.

[9] K. Locher, C. R. Belanger, E. Eckbo, M. Caza, B. Velapatino, and M. K. Charles, "Automated 16S sequencing using an r-based analysis module for bacterial identification," Microbiol. Spectrum, vol. 10, no. 2, pp. e00408-22, 2022.

[10] A. Bazzi, B. M. Masini, A. Zanella, and I. Thibault, "On the performance of IEEE 802.11 p and LTE-V2V for the cooperative awareness of connected vehicles," IEEE Trans. Veh. Technol., vol. 66, no. 11, pp. 10419–10432, 2017.

[11] A. M. Abdo, A. Fouda, A. M. Eid, N. M. Fahmy, A. M. Elsayed, A. M. A. Khalil, and A. M. Soliman, "Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) by Pseudomonas aeruginosa and Their Activity Against Pathogenic Microbes and Common House Mosquito, Culex pipiens," Materials, vol. 14, no. 22, p. 6983, 2021.

[12] M. Barsainya and D. P. Singh, "Green Synthesis of Zinc Oxide Nanoparticles by Pseudomonas aeruginosa and Their Broad-Spectrum Antimicrobial Effects," J. Pure Appl. Microbiol., vol. 12, no. 4, pp. 2123–2134, 2018.

[13] I. Y. Tafinta, K. Shehu, H. Abdulganiyyu, A. M. Rabe, and A. Usman, "Isolation and Identification of Fungi Associated With the Spoilage of Sweet Orange (Citrus sinensis) Fruits in Sokoto State," Nigerian J. Basic Appl. Sci., vol. 21, no. 3, pp. 193–196, 2013.

[14] H. Anton, H. Al-Ghoshae, M. Chandra, M. F. Baobaid, T. Aung, H. Hazman, and A. Al-Kabsi, "Formalin-Resistant Fungi Isolated From Cadavers at a Medical School's Dissection Hall in Malaysia," Asian J. Med. Health Sci., vol. 5, no. 1, p. 56, 2022.

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

[15] A. Es-Haghi, M. E. Taghavizadeh, M. Sharifalhoseini, M. Baghani, E. Yousefi, A. Rahdar, and F. Baino, "Application of Response Surface Methodology for Optimizing the Therapeutic Activity of ZnO Nanoparticles Biosynthesized From Aspergillus niger," Biomimetics, vol. 6, no. 2, p. 34, 2021.

[16] A. Shamim, M. B. Abid, and T. Mahmood, "Biogenic Synthesis of Zinc Oxide (ZnO) Nanoparticles Using a Fungus (Aspergillus niger) and Their Characterization," Int. J. Chem., vol. 11, no. 2, pp. 119–126, 2019.

[17] A. E. Mekky, A. A. Farrag, A. A. Hmed, and A. R. Sofy, "Preparation of Zinc Oxide Nanoparticles Using Aspergillus niger as Antimicrobial and Anticancer Agents," J. Pure Appl. Microbiol., vol. 15, pp. 1547–1566, 2021.

[18] S. Jacob, R. Bharathkumar, and G. Ashwathram, "Aspergillus niger Mediated Synthesis of ZnO Nanoparticles and Their Antimicrobial and In Vitro Anticancerous Activity," World J. Pharm. Res., vol. 3, no. 2, pp. 3044–3054, 2014.

[19] G. Baskar, J. Chandhuru, K. S. Fahad, and P. A. S. Praveen, "Mycological Synthesis, Characterization and Antifungal Activity of Zinc Oxide Nanoparticles," Asian J. Pharm. Technol., vol. 3, no. 4, pp. 142–146, 2013.

[20] P. P. George, "Value-Addition of Pomegranate Fruit Peel Biowaste: Microwave-Mediated Green Synthesis, Characterization and Antimicrobial Activity of Zinc Oxide Nanoparticles," Asian Basic Appl. Res. J., pp. 17–24, 2022.

[21] S. R. Senthilkumar and T. Sivakumar, "Green Tea (Camellia sinensis) Mediated Synthesis of Zinc Oxide (ZnO) Nanoparticles and Studies on Their Antimicrobial Activities," Int. J. Pharm. Pharm. Sci., vol. 6, no. 6, pp. 461–465, 2014.

[22] D. B. Stensen, R. A. N. Cñadas, L. Småbrekke, K. Olsen, C. S. Nielsen, K. Svendsen, and A. S. Furberg, "Social Network Analysis of Staphylococcus aureus Carriage in a General Youth Population," Int. J. Infect. Dis., vol. 123, pp. 200–209, 2022.

[23] J. G. Collee, "A Dreadful Challenge," The Lancet, vol. 347, no. 9006, pp. 917–918, 1996.

[24] J. MacFaddin, "Deoxyribonuclease (DNase) and Thermos Nuclease (Tnase) Tests," Biochemical Tests for Identification of Medical Bacteria, 3rd ed., Philadelphia, PA: Lippincott Williams and Wilkins, 2000, pp. 136–159.

[25] C. Brennan, H. Momota, D. Hambardzumyan, T. Ozawa, A. Tandon, A. Pedraza, and E. Holland, "Glioblastoma Subclasses Can Be Defined by Activity Among Signal

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Transduction Pathways and Associated Genomic Alterations," PLoS One, vol. 4, no. 11, p. e7752, 2009.

[26] P. Lakshmi, A. Bharadwaj, and R. K. Srivastava, "Molecular Detection and Identification of Bacteria in Urine Samples of Asymptomatic and Symptomatic Pregnant Women by 16S rRNA Gene Sequencing," Archives of Clin. Infect. Dis., vol. 15, no. 3, 2020.

[27] R. Srinivasan, U. Karaoz, M. Volegova, J. MacKichan, M. Kato-Maeda, S. Miller, and S. V. Lynch, "Use of 16S rRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens," PLoS One, vol. 10, no. 2, p. e0117617, 2015.

[28] H. T. M. Al-Mousawi, M. I. AL-Taee, and A. T. Al-Musawi, "Evaluation of the Effects of Fe3O4 Nanoparticles on Biofilm Gene Expression Patterns in Acinetobacter baumannii Using Quantitative Real-Time Polymerase Chain Reaction Assay," J. Global Pharma Technol., vol. 11, no. 3, pp. 579–589, 2019.

[29] R. H. A. Al-Saeedi and R. H. Raheema, "Molecular Diagnosis of Some Virulence Genes in Pseudomonas aeruginosa Clinical Isolates in Wasit Province," Indian J. Public Health, vol. 10, no. 4, 2019.

[30] P. Basnet, T. I. Chanu, D. Samanta, and S. Chatterjee, "A Review on Bio-Synthesized Zinc Oxide Nanoparticles Using Plant Extracts as Reductants and Stabilizing Agents," J. Photochem. Photobiol. B: Biol., vol. 183, pp. 201–221, 2018.

[31] Q. N. Al-Hajjar and H. T. M. Al-Mousawi, "Sero-Prevalence of Helicobacter pylori Among Staff and Students in Faculty of Pharmacy," Indian J. Forensic Med. Toxicol., vol. 13, no. 4, p. 2, 2019.

[32] A. K. Mandal, S. Katuwal, F. Tettey, A. Gupta, S. Bhattarai, S. Jaisi, and N. Parajuli, "Current Research on Zinc Oxide Nanoparticles: Synthesis, Characterization, and Biomedical Applications," Nanomaterials, vol. 12, no. 17, p. 3066, 2022.

[33] K. Koupadi, S. C. Boyatzis, M. Roumpou, N. Kalogeropoulos, and D. Kotzamani, "Organic Remains in Early Christian Egyptian Metal Vessels: Investigation With Fourier Transform Infrared Spectroscopy and Gas Chromatography–Mass Spectrometry," Heritage, vol. 4, no. 4, pp. 3611–3629, 2021.

[34] M. J. Mustafa, M. Al-Bderee, and H. T. M. Al-Mousawi, "Biosynthesis Nanoparticles and Molecular Study to Detect Bacterial Isolates From Patients With Diabetic Foot

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.57

Ulcers in Babylon Province," J. Pharm. Negative Results, vol. 13, no. 5, pp. 391–395, 2022.

[35] M. F. Hamza, K. A. Salih, A. H. Adel, Y. E. Zayed, Y. Wei, J. Liang, and E. Guibal, "Sulfonic-Functionalized Algal/PEI Beads for Scandium, Cerium and Holmium Sorption From Aqueous Solutions (Synthetic and Industrial Samples)," Chem. Eng. J., vol. 403, p. 126399, 2021.

[36] M. Ebadi, M. R. Zolfaghari, S. S. Aghaei, M. Zargar, M. Shafiei, H. S. Zahiri, and K. A. Noghabi, "A Bio-Inspired Strategy for the Synthesis of Zinc Oxide Nanoparticles (ZnO NPs) Using the Cell Extract of Cyanobacterium Nostoc sp. EA03: From Biological Function to Toxicity Evaluation," RSC Adv., vol. 9, no. 41, pp. 23508–23525, 2019.

[37] S. Sarwar, S. Chakraborti, S. Bera, I. A. Sheikh, K. M. Hoque, and P. Chakrabarti, "The Antimicrobial Activity of ZnO Nanoparticles Against Vibrio cholerae: Variation in Response Depends on Biotype," Nanomedicine: Nanotechnol., Biol. Med., vol. 12, no. 6, pp. 1499–1509, 2016.