GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

A Comparative study of the effect of zinc oxide and silver nanoparticles against antibiotic-resistant *Pseudomonas aeruginosa*

Khalida Jhalil Ibraheem *

Department of Science,College of Basic Education, Mustansiriyah University, Iraq.

GSC Biological and Pharmaceutical Sciences, 2025, 32(02), 001-006

Publication history: Received on 16 June 2025; revised on 29 July 2025; accepted on 01 August 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.32.2.0296

Abstract

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium that causes serious infections in immunocompromised patients and is known for its high capacity to develop resistance to multiple antibiotics, making infections difficult to treat. The study aimed to investigate the prevalence of multiple antibiotic-resistant *Pseudomonas aeruginosa* species that cause many diseases, and to estimate the impact of nanoparticles on them and their use as alternatives or adjuncts to antibiotic treatment. A total of 182 clinical samples were collected, including urinary tract infections, wound swabs, pus, burns, and tonsillitis, from patients arriving at Al-Kindi Hospital in Baghdad between March to September 2024. Twenty-nine strains of *Pseudomonas aeruginosa* (16%) were identified based on morphological and cultural characteristics, biochemical tests, and the APi20E test. These strains were tested for their sensitivity to 10 types of antibiotics. The species showed high resistance to Ampicillin and Amoxicillin (100%), and the least resistance to Ciprofloxacin (35%). Nine strains with high resistance to antibiotics were selected, and the minimum inhibitory concentration (MIC) was determined against the antibiotics under study. In addition, some nanoparticles of different sizes (Ag20, 90nm, ZnO20, 30, 50~150, nm. The results showed that the MIC for Ag20, 90nm was 650-2600 µg/ml, while for ZnO20nm, the MIC values ranged between 81.25 -2600 µg/ml, while the MIC for ZnO30, 50~150nm was between 325- 2600 (µg/ml). The synergistic effect between antibiotics and nanomaterials showed high effectiveness in inhibiting the growth of *Pseudomonas aeruginosa*.

Keywords: Multi-resistant to antibiotics; *Pseudomonas aeruginosa*; Zinc oxide and silver nanoparticles

1. Introduction

Nanoparticles are a distinct class of fine materials that can be produced with dimensions ranging from 1 to 100 nanometers. The small size and dimensions of these materials allow them to behave differently from conventional large sized materials with dimensions greater than 100 nanometers. This means that nanoparticles have electrical and magnetic properties that differ significantly from larger particles of the same compounds [1]. Metallic nanoparticles can be obtained by biological, physical, and chemical methods, as several types of bacteria and fungi have the ability to produce metal nanoparticles with antimicrobial properties [2]. It has been known since ancient times that silver and its compounds have various effects against microorganisms and viruses. This is due to the small size of its particles, less than 5 nanometers, which increases their surface area and thus leads to a tendency to migrate to the outer surface of the nanoparticles, increasing their chemical activity and increasing the production of reactive oxygen species, including the formation of free radicals. [3] Silver nanoparticles also have the ability to cross living membranes, such as skin affected by eczema or burns, and reach the bloodstream, leading to increased oxidative stress, the production of cytokines, inflammation, and ultimately cell death [4]. Zinc oxide nanoparticles are among the most popular metal oxides, possessing numerous properties, most notably semiconductor properties and antibacterial activity, which has attracted significant interest as an antibacterial treatment [5]. They have stronger antibacterial activity than zinc oxide, due to the significantly increased surface area of the particles. Zinc oxide nanoparticles rival other metal oxide

* Corresponding author: Khalida J. Ibraheem

nanoparticles in their antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria [6]. Pathogenic bacteria characterized by multidrug resistance and possessing plasmids have become a major problem for humans, particularly as these bacteria are resistant to a number of antibiotics and can continue to grow in the body despite antibiotic treatment [7]. Adding antibiotics to nanoparticles has shown synergistic effects against bacteria. Using the particles alone has shown a 40% inhibition of their growth, and the percentage increased significantly with the addition of antibiotics [8]. Accordingly, the study aimed to investigate the prevalence of multiple antibiotic-resistant *Pseudomonas aeruginosa* species that cause many diseases, and to estimate the impact of nanoparticles on them and their use as alternatives or adjuncts to antibiotic treatment.

2. Material and Methods

A total of 182 samples were collected from patients attending Al-Kindi Hospital in Baghdad city during the period from March to September 2024. The samples included urine, wound swabs, pus, burns, and tonsillitis. *Pseudomonas aeruginosa* was isolated and identified from the different samples according to [9,10] based on morphological, cultural, and biochemical characteristics, and the API-20E system prepared by BioMerieux according to its specifications [11].

Antibiotic sensitivity testing was performed using the disc diffusion method. Ten antibiotics were used: Ampicillin (AM/10 µg), Amoxicillin (AX/25 µg), Erythromycin (E/15 µg), Ciprofloxacin (CIP/5 µg), Chloramphenicol (C/30 µg), Cefotaxime (CTX/30 µg), Nitrofurantoin (NF/300 µg), Co-Trimoxazole (COT/25 µg), Doxycycline (DO/30µg), Ceftriaxone (CRO/30µg). Using solid Mueller-Hunton medium, the media were incubated at 37°C for 18 hours. The results were compared with international measurements, as stated in [12]. The broth dilution method was used for quantitative assessment to demonstrate the effectiveness of antibiotics and nanoparticles. A 96-well microtiter plate method was used, and the assay was conducted according to [13,14] as follows:

- 100 microliters of Mueller-Hunton broth were added to each well.
- 100 microliters of the previously prepared antibody at a concentration of 1024 µg/ml were added to well 1 of row A. The antibody was mixed by vibrating up and down for 6-8 minutes, resulting in a double concentration of 512 µg/ml. • Transfer 100 microliters from well 1 to well 2 and mix with the same movement. Thus, the diluent concentration became 256 µg/mL. • The process was repeated until reaching well 10, after which 100 microliters were withdrawn and discarded, resulting in a concentration of (512, 256, 128, 64, 32, 16, 8, 4, 2, 1) µg/ml.
- 5 microliters of 24-hour-old bacterial suspension were added to each well.
- Only broth and bacterial suspension were added to well 11, creating a positive control.
- Well 12 became a negative control, containing only broth and antibody.
- Another antibody was added to row B, and so on for the other rows.
- The plate was covered and incubated at 37°C for 18-24 hours.
- Visually check for growth, and then note the MIC (minimum inhibitory concentration) of the antibody by observing the turbidity of the medium.

As for the nanoparticles, the same method was used, using a concentration of 5200 µg/ml, previously prepared in hole 1 of row A. This resulted in concentrations of (2600, 1300, 650, 325, 162.5, 81.25, 40.6, 20.3, 10.15, 5.07) µg/ml. The synergistic effect of silver particles and zinc oxide, individually with antibiotics was estimated by adding 50 microliters of the particles at their minimum inhibitory concentration with 50 microliters of antibiotics at their inhibitory concentration to 5 mm diameter holes after inoculating the surfaces of the plates with the pure species under study. The plates were incubated at 37°C for 24 hours, and the diameters of inhibition around the holes were measured (in mm). The process was repeated twice, and the average was taken [15].

3. Results and Discussion

182 clinical samples were collected from patients, including urinary tract infections, wound swabs, pus, burns, and tonsillitis. Twenty-nine strains of *Pseudomonas aeruginosa* (16%) were identified based on morphological and cultural characteristics, biochemical tests, and the APi20E test. Figure (1) shows the percentage of *Pseudomonas aeruginosa* isolated from different clinical sources, with 13 isolates from burns dominating (45%).

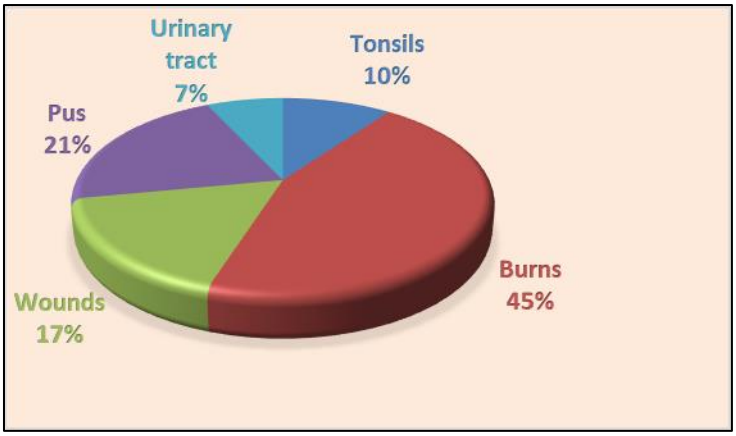

Figure 1 The percentage of *Pseudomonas aeruginosa* isolated from different clinical sources

The sensitivity of the isolated bacteria to 10 antibiotics was studied by measuring the zone of inhibition diameter and comparing the results with what was reported in [16]. The results in Figure (2) showed that there was a variation in the resistance of the isolates under study to the antibiotics used. The bacteria showed high resistance to the antibiotics Ampicillin and Amoxicillin at 100%, consistent with many researchers [17]. They were followed by the antibiotics Nitrofurantoin, Co-Trimoxazole, Ceftriaxone, and Erythromycin at 88%, 84%, 77%, and 71%, respectively. The results also showed high resistance to some antibiotics, including Cefotaxime and Doxycycline at 67% and 61%, respectively. This percentage is considered normal rate to the indiscriminate use of antibiotics. This result is consistent with [18], where a high percentage of resistance to the antibiotic Cefotaxime was found among *Pseudomonas aeruginosa* isolates. the resistant to Chloramphenicol at a moderate rate of 55%, while the lowest resistance was to Ciprofloxacin 35%. Ciprofloxacin has shown a broad effect against Gram-negative bacteria, as recorded by [19], as it inhibits the activity of the DNA gyrase enzyme [20].

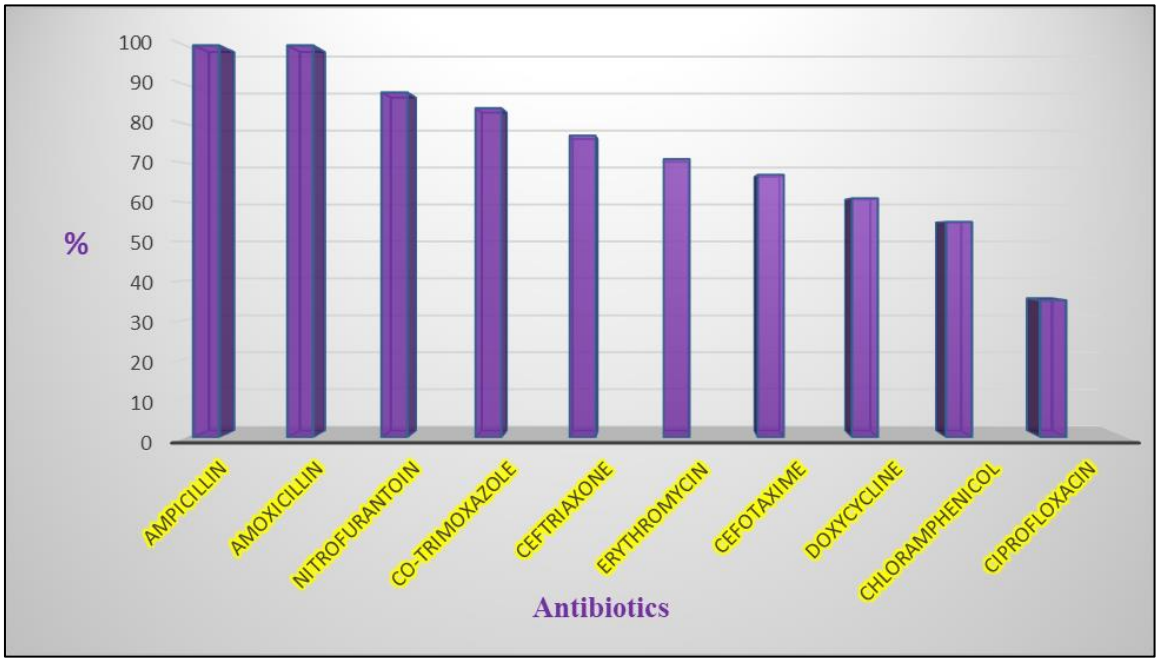

Figure 2 Resistance rates of *Pseudomonas aeruginosa* isolates to the antibiotics under study

9 strains with high resistance to antibiotics were selected and the minimum inhibitory concentration (MIC) was determined against the antibiotics under study. As shown in Table 1, the breakpoint described by [12] was adopted as the basis for calculating the response. The results showed that all isolates showed complete resistance to all antibiotics and gave high MIC values compared to the breakpoint, with the exception of the sensitivity of all isolates to the antibiotics Cefotaxime and Ceftriaxone, and 8 isolates were sensitive to Nitrofurantoin, where the MIC values ranged between 2-64 µg/ml, except for one isolate that was resistant, with an MIC value of 128 µg/ml.

Table 1 Minimum inhibitory concentration (MIC) of different antibiotics against *Pseudomonas aeruginosa* isolates

Isolate number				MIC						
	AM	**AX**	**NF**	**COT**	**CRO**	**E**	**CTX**	**DO**	**C**	**CIP**
P1	**512**	**256**	**128**	**256**	**64**	**16**	**2**	**16**	**64**	**4**
P2	**128**	**256**	**64**	**64**	**32**	**128**	**16**	**32**	**32**	**16**
P3	**512**	**128**	**32**	**8**	**16**	**256**	**8**	**4**	**128**	**32**
P4	**256**	**128**	**16**	**64**	**8**	**64**	**16**	**32**	**64**	**4**
P5	**128**	**128**	**8**	**16**	**4**	**64**	**8**	**8**	**8**	**2**
P6	**512**	**256**	**16**	**64**	**2**	**128**	**64**	**32**	**32**	**32**
P7	**128**	**512**	**4**	**32**	**8**	**16**	**64**	**128**	**128**	**4**
P8	**128**	**512**	**32**	**8**	**32**	**256**	**32**	**64**	**32**	**8**
P9	**512**	**256**	**2**	**32**	**16**	**128**	**8**	**16**	**8**	**32**

The minimum inhibitory concentration (MIC) for silver nanoparticles (Ag-NP) with a size of 20 and 90 nm on *Pseudomonas aeruginosa* isolates is shown in Table 2. It was between 650 - 2600 µg/ml, indicating that *Pseudomonas aeruginosa* isolates are more resistant and require a higher concentration of silver nanoparticles to affect bacterial growth. These results are similar to what was reported by [21]. The minimum inhibitory concentration (MIC) for zinc oxide nanoparticles (ZnO-NP) with a size of 20 nm on *Pseudomonas aeruginosa* isolates was between 81.25- 2600 µg/ml, while the minimum inhibitory concentration (MIC) for zinc oxide nanoparticles (ZnO-NP) with a size of 30 nm was between325- 1300 µg/ml while the minimum inhibitory concentration (MIC) for zinc oxide nanoparticles (ZnO-NP) with a size of 50~150nm was between 325-2600 µg/ml. The antibiological activity of zinc oxide nanoparticles against *Pseudomonas aeruginosa* bacteria increases with the decrease in particle size [22].

Table 2 Minimum inhibitory values (MIC) of different nanoparticles against *Pseudomonas aeruginosa* isolates

Isolate number			MIC		
	ZnO20nm	**ZnO30nm**	**ZnO50~150nm**	**Ag20nm**	**Ag90nm**
P1	1300	650	1300	2600	650
P2	1300	1300	650	2600	2600
P3	650	1300	650	1300	650
P4	650	1300	1300	1300	2600
P5	81.25	1300	650	650	650
P6	2600	1300	2600	325	650
P7	1300	325	650	2600	2600
P8	325	650	325	1300	1300
P9	650	650	325	2600	625

Table (3) shows the effect of the relationship between antibiotics and nanoparticles on *Pseudomonas aeruginosa* bacteria, where the antibiotics (E, CIP, CTX, DO, CRO) showed an increase in the average diameter of the inhibition zone when mixed with the nanoparticles used in the study and their different sizes. The study [23] showed the emergence of a synergistic effect when the combination of silver nanoparticles and the antibiotic chloramphenicol against *Pseudomonas aeruginosa* bacteria is inconsistent with [24]. However, it is consistent with [25], which stated that antibiotics showed a synergistic effect with nano zinc oxide against bacteria producing extended-spectrum beta-lactamase enzymes. The combination of nano zinc oxide and antibiotics increases the permeability of the cell membrane, thus leading to protein leakage from the bacterial membrane.

Table 3 Effect of the combination of antibiotics and nanoparticles on the species *Pseudomonas aeruginosa*

Antibiotics	Antibiotic effect alone	Antimicrobial effect with nanoparticles					The effect of nanoparticles alone				
		ZnO2 0nm	ZnO3 0nm	ZnO50~150nm	Ag20 nm	Ag90 nm	ZnO2 0nm	ZnO3 0nm	ZnO50~150nm	Ag20 nm	Ag90 nm
AM	0	0	0	0	0	0	0	0	0	0	0
AX	0	0	0	0	0	0					
NF	0	0	0	0	0	0					
COT	0	0	0	0	0	0					
CRO	10	13	10	11	7	8					
E	8	13	0	0	0	0					
CTX	9	9	11	10	11	11					
DO	8	7	8	0	8	0					
C	0	0	0	0	0	0					
CIP	14	17	16	14	18	17					

4. Conclusion

We conclude from this study that nano zinc oxide is more effective against antibiotic-resistant bacteria than silver nanoparticles. The bacterial isolates were resistant to several groups of antibiotics, including penicillins and cephalosporins, while they were sensitive to ciprofloxacin. The synergistic effect of the antibiotics and nanoparticles used in the study against the bacterial isolates varied. Ciprofloxacin, Ceftriaxone, and Cefotaxime, when combined with the nanoparticles used in the study, had the highest effect against the bacterial isolates.

Compliance with ethical standards

Acknowledgments

My thanks and appreciation for the management of the Al-Mustansiriyah university.

Disclosure of conflict of interest

There are no conflicts of interest for any of the authors.

Statement of informed consent

This study was done with the consent of the patients or the parents.

References

[1] Dahal P, Shrestha M, Maharjan M, Parajuli R. Multidrug-resistant Pseudomonas aeruginosa and its coexistence with β-lactamases at a tertiary care hospital in a low-resource setting: a cross-sectional study with an association of risk factors. Ther Adv Infect Dis. 2025 Jun 18; 12:2049.

[2] Elfadadny A, Ragab RF, AlHarbi M, et al. antimicrobial resistance of *Pseudomonas aeruginosa*: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. Front Microbiol 2024; 15: 1374466.

[3] Hafiz TA, Bin Essa EA, Alharbi SR, et al. Epidemiological, microbiological, and clinical characteristics of multi-resistant *Pseudomonas aeruginosa* isolates in King Fahad Medical City, Riyadh, Saudi Arabia. Trop Med Infect Dis 2023; 8(4): 205.

[4] Hafiz TA, Bin Essa EA, Alharbi SR, et al. Epidemiological, microbiological, and clinical characteristics of multi-resistant *Pseudomonas aeruginosa* isolates in King Fahad Medical City, Riyadh, Saudi Arabia. Trop Med Infect Dis 2023; 8(4): 205.

[5] Sando E, Suzuki M, Ishida M, et al. Definitive and indeterminate *Pseudomonas aeruginosa* infection in adults with community-acquired pneumonia: a prospective observational study. Annals Am Thoracic Soc 2021; 18(9): 1475–1481.

[6] Reynolds D, Kollef M. The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: an update. Drugs 2021; 81(18): 2117–2131.

[7] Mirzaei B, Bazgir ZN, Goli HR, et al. Prevalence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and Acinetobacter baumannii isolated in clinical samples from Northeast of Iran. BMC Res Notes 2020; 13: 380.

[8] Duran, N., et al, "Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action", Journal of the Brazilian Chemical Society. 21 (6):949-959 (2010).

[9] Funke, G., Von Graevenitz, A., Glarridge, J.E. and Betnard, K.A., "Clinical microbiology of coryneform bacteria", Clinical Microbiology Reviews, Vol. 10(1):125-159, American Society for Microbiology, (1997).

[10] Gillespie S. H. and Hawkey P. M.," Principles and Practice of Clinical Bacteriology ".2nd ed., John Wiley and Sons, Ltd. England.P:457(2006).

[11] Winn, W., Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P. and Woods, G., "Koneman's Color Atlas and Textbook of Diagnostic Microbiology", 6th ed., Lippincott Williams and Wilkins, U.S.A. pp.318-321(2006).

[12] CLSI, (Clinical & Laboratory Standards institute)." Performance Standard for Antimicrobial Susceptibility Testing", Scientific Information Database (SID) journal 31(1), 1-163(2011).

[13] D. Amsterdam, "Susceptibility testing of antimicrobials in liquid media. In: Loman V., ed. Antibiotics in Laboratory Medicine", 4th ed.Williams and Wilkins, Baltimore, MD. Japan, p.52-111(1996).

[14] Saginur, R., Denis, M.S., Ferris, W., Aaron, S.D., Chan, F., Lee, C. and Ramotar, K., "Multiple combination bactericidal testing of Staphylococcal Biofilms from implant-associated infections". Antimicrobial Agents Chemotherapy, 50(1): 55-61(2006).

[15] Egorove, N.S., "Antibiotic a sientific approach", Mir publishers, Moscow, (1985).

[16] Dannel, F., Hall, L., Duke, B., Cur, D. and Livermore, D., "OXA-17, further extended spectrum variant of OXA-10 ß-Lactamase, isolated from *Pseudomonas aeruginosa*". Antimicrobial agents and chemotherapy, 43(6): 1362-1366(1999).

[17] Mouneimne, H., Robert, J., Jarlier, V. and Cambua, E., "Type II topoisom erase mutation in ciprofloxacin resistant strains of P. aeruginosa", Antimicrobial agents and chemotherapy, 43 (1): 62 -66(1999).

[18] Fluit, A.C., Visser, M.R., and Schmitz, F.J., "Molecular detection of antimicrobial resistance", Clinical Microbiology Reviews, 14 (4): 836-871(2001).

[19] Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B. and Yacaman, M.J., "The Bactericidal effect of silver nanoparticles", Nanotechnology, 16(10):2346–2353(2005).

[20] Padmavathy, N. and Vijayaraghavan, R., "Enhanced bioactivity of ZnO nanoparticles-an antimicrobial study", Science and technology of advanced materials, 9(3):1-7 (2008).

[21] Meruvu, H.; Vangalapati, M.; Chippada, S. and Bammidi, S., "Synthesis and characterization of ZnO nanoparticles and antimicrobial activity against Bacillus subtitles and Escherichia coli", Rasayan journal Chemical, 4(1): 217-222(2011).

[22] Hwang, I.; Hwang, J. H.; Choi, H.; Kim, K. and Lee, D.G.," Synergistic effects between silver nanoparticles and antibiotics and the mechanisms involved", Journal of Medical Microbiology, 61(12): 1719–1726(2012).

[23] Hari, N.; Thomas, T.K. and Nair, A.J.," Comparative Study on the Synergistic Action of Garlic Synthesized and Citrate Capped Silver Nanoparticles with β-Penem Antibiotics", Hindawi Publishing Corporation, ISRN Nanotechnology, Article ID 792105, 6 pages, Volume 2013 (2013).

[24] Bhande, R.M.; Khobragade, C.N.; Mane, R.S. and Bhande, S.,"Enhanced synergism of antibiotics with zinc oxide nanoparticles against extended spectrum b-lactamase producers implicated in urinary tract infections". Journal of nanoparticle research, 15(1):1-13 (2013).

[25] Horcajada Juan P., Montero M., Oliver A., Sorlí L., Luque S., Gómez-Zorrilla S., Benito N., Grau S. Epidemiology and Treatment of Multidrug-Resistant and Extensively Drug-Resistant *Pseudomonas aeruginosa.* Infections. Clin. Microbiol. Rev. 2019;32: e00031-19.