ARTICLE / INVESTIGACIÓN

Inhibitory effect of Titanium dioxide (TiO_2) nanoparticles and their synergistic activity with antibiotics in some types of bacteria

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Abstract: Titanium dioxide nanoparticles (TiO_2 NPs) were studied as antibacterial agents at different concentrations against clinical and environmental bacterial isolates without UV or photocatalytic activation. Five TiO_2 NPs concentrations (20µg/ml,50µg/ml, 100µg/ml,500µg/ml and 1000µg/ml) were studied against 15 bacterial species:10 clinical isolates and 5 environmental isolates) compared with antibiotics Amikacin(AK)and Levloxacin(LEV).Only500µg/ml concentration of TiO_2 NPs was active against 7 bacterial isolates (3 clinical and 4 environmental), and 1000µg/ml concentration of TiO_2 NPs was effective against 9 isolates (6 clinical and 3 environmental). These concentrations were mixed with the antibiotics Levloxacin LEV and Amikacin AK to investigate the possibility of synergistic activity against studied bacteria. Bacterial isolate's response or sensitivity to the antibiotic and TiO_2 NPs mixture was varied; AK plus 500µg/ml TiO_2 NPs concentration showed increased inhibitory activity against 7 isolates (3 clinical, 4 environmental) and 1000µg/ml TiO_2 NPs mixed with AK showed increased inhibition activity against one environmental bacterial isolates, where Ak mixed with 500 and Ak plus 1000 µg/ml showed the same effect as the antibiotic alone or less.LEV antibiotic shows no difference in the effect on all 9 bacteria (7 clinical and 2 environmental), while LEV mixed with 500 µg/ml have increased inhibition zones on 4 bacteria (2 clinical, 2 environmental) , and LEV mixed with 1000µg/ml have higher effect than the antibiotic alone on three isolates (2 clinical,1 environmental).

Key words: Antibiotic, titanium nanoparticles dioxide, antibacterial.

Introduction

The mechanical properties of titanium-based implants have improved significantly in recent years thanks to using TiO_2 NPs in medical devices. Overcome the bio inertness of the raw metals, corrosion resistance and the rate at which metal ions are released (to prevent aseptic loosening of the implant)[1].

In the early stages of the "race for the surface," the implant may get infested with bacteria. Aseptic loosening of implants and biomaterial-centered infections (BCI) play a significant role in prosthetic implant failure and infection of the implant itself, making them a severe medical problem[2].

In addition to its photocatalytic properties, TiO_2 NPs can be used for water separation, energy production, air and water purification and surface sterilizing, organic compound synthesis, and pollution reduction(TiO_2 can be used as an adsorbent for environmental pollutants, where it deals with air pollutants such as dust and dust and adsorbs them on the surfaces on which they fall. As for water pollutants, they can be used as a precipitant for contaminants that contain water.)[3] Nanoparticles (NPs) are also employed to make medications, detect infections, proteins, and tumors, and separate and purify biological components and cells[4,5]. Titanium dioxide (TiO_2) inorganic nanoparticles have been used for decades due to their non-toxicity, ease of production, and low cost. Furthermore, it may have a universal bactericidal mechanism[6]. The morphological characteristics of titanium dioxide NPs significantly impact their applications[7]. Rutile, anatase, and brookite are all types of TiO_2 NPs that can be found in nature. Of the three types of photocatalysts mentioned, anatase NPs are the most commonly used[8,9].

Many people are interested in the antibacterial characteristics of TiO_2 in the food industry. TiO_2 has been declared nontoxic by the FDA in the United States. TiO_2 has been Many people are interested in the antibacterial characteristics of TiO_2 in the food industry right now. TiO_2 has been declared nontoxic by the FDA in the United States[10]. In addition to its fungicidal activity, TiO_2 NPs have been demonstrated to exhibit bactericidal effects on bacteria such as *E. coli, Staphylococcus aureus*, and *Pseudomonas putida*[11,12]. There has been some interest in food packaging with TiO_2-coated or incorporated TiO_2[13,14]. For food packaging, antimicrobial agents combat germs and enhance traditional packaging functions, such as shelf life extension, quality preservation, and safety assurance[15]. Using an antibacterial agent helps to reduce the spread of hazardous germs and keep food fresh, as shown in figure (1)[16,17]

The anatase form of titanium dioxide nanoparticles (without UV activation or photocatalytic activation) was examined for its antibacterial activity against pathogenic and environmental Gram-positive and Gram-negative bacteria to assess its application as a new antibacterial approach or for ecological health.

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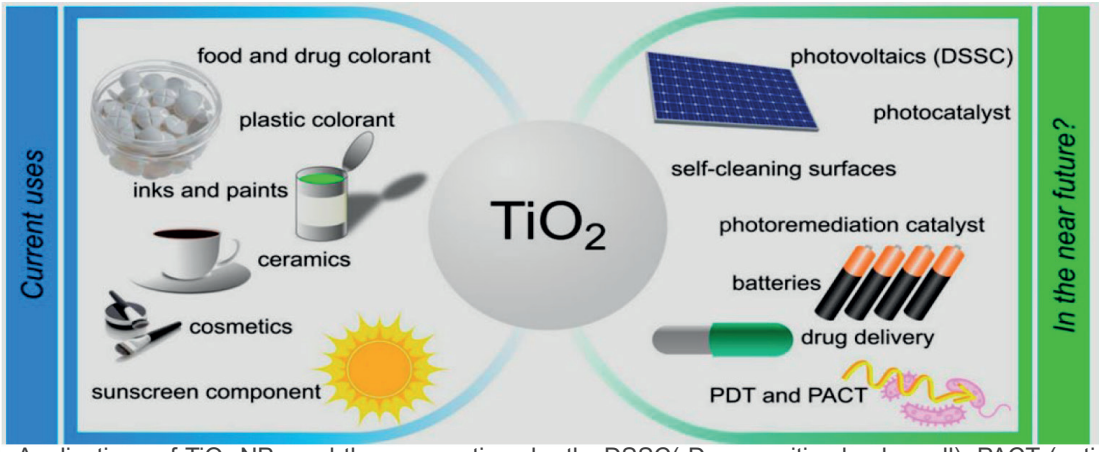

Figure 1. Applications of TiO_2 NPs and the perspective shortly. DSSC(Dye-sensitized solar cell); PACT (antimicrobial photodynamic therapy); PDT (photodynamic therapy)[18].

Materials and methods

Nanoparticles

The Canadian company MK impex corp supplies commercial TiO2 nanoparticles with a size of 100nm. Were used in this study.

Bacterial Isolates

A total of 15 bacterial isolates were used, which included 7 clinical isolates obtained from wound and urine infections and 6 environmental isolates from water, as shown in table (1).

All isolates were identified by standard microbiological procedures (Gram staining, colonial morphology, catalase test, cytochrome oxidase reaction, motility, and other biochemical tests), which were carried out depending on Bergey's manual of systematic Bacteriology, also by Vitek 2 system[19].

Use of TiO_2 NPs as a bactericidal inhibitor of

For testing the antibacterial activity, five concentration of TiO2 NPs were prepared by dissolving the NPs powder in deionized distilled water[20]; different dilution was prepared, including (1000 µg/ml,500 µg/ml,100µg/ml, 50 µg/ml,20 µg/ml)

This preparation was mixed using a vortex for 3 minutes to obtain homogeneous dilution. Whatman no. 1 filter paper discs were saturated with each concentration of TiO_2 NPs and used in the sensitivity test.

Antibiotic Amikacin AK (10 mcg) and Lefloxacin LEF (5 mcg) supplied by Bioanalyzer (turkey) were used as positive control for each bacterium.

Bacterial inoculums were prepared by Direct Colony Suspension Method (1-3). Isolation colonies were selected and transferred to a tube of nutrient broth mixed well using a vortex; the bacterial no was fixed at 1.5×10^8 by comparing the turbidity with the 0.5 McFarland standard[21]. Mueller-Hinton agar plates were inoculated with the suspension of each bacterial strain, then five TiO_2 NPs discs, as well as the antibiotic discs, were distributed unit formally on the agar surface, then incubated at 37°C for (20-24) h. Inhibition zones were measured to monitor the effects of TiO_2 NPs on bacterial growth[22]. Positive results were scored when a zone of inhibition was observed around the discs after incubation. The experiments were performed in triplicate to obtain means values for each bacterial isolates[9].

Synergistic effects between the active concentrations of TiO_2 NPs and the antibiotics against bacteria

The conc.1000 µg/ml and 500 µg/ml were chosen and mixed with the standard antibiotic discs by adding 250 µl from each concentration to 15 antibiotic discs after the experiment in 15 minutes.The antibiotic sensitivity disc was done according to CLSI. Each experiment was repeated three times.

1-	*Aeromonas spp.*	11-	*Staphylococcus aureus 1*
2-	*Staphylococcus aureus 16*	12-	*Staphylococcus aureus 2*
3-	*Klebsiella Oxytoca*	13-	*Staphylococcus aureus 3*
4-	*Enterobacter cloacae*	14-	*Escherichia coli 1*
5-	*burkholderia cepacia*	15-	*Pseudomonas aeruginosa 3*
6-	*Escherichia coli 4*		
7-	*Escherichia coli 3*		
8-	*Pseudomonas aeruginosa*		
9A	*Pseudomonas aeruginosa 2*		
10	*Pseudomonas aeruginosa1*		

Table 1. Bacterial isolates were used in this study.

Results

The activity of five concentrations of TiO_2 nanoparticles is shown in table (2), as well as control antibiotics AK and LEV against 15 bacterial isolates from clinical and environmental sources. The concentrations (20, 50, 100) µg/ml didn't show any antibacterial against all bacteria, while the concentration of 500µg/ml of TiO2 nanoparticles shows inhibitory activity against 7 isolates (3 clinical and 4 environmental including *Aeromonas spp., Klebsiella Oxytoca, Pseudomonas aeruginosa* 1.

Staphylococcus aureus 1, Staphylococcus aureus 3, Escherichia coli 1, Pseudomonas aeruginosa 3), the 1000µg/ml showed antibacterial activity against 9 isolates (6 clinical isolates including *Aeromonas spp., Klebsiella oxytoca, Enterobacter cloacae, Burkholderia cepacia, Escherichia coli 4, Escherichia coli 3* and 3 environmental isolates including *Stapylococcus aureus 1, Staphylococcus aureus 3, Escherichia coli 1*).

The inhibitory effect of these two concentrations differs against the different bacterial isolates used, and it also concerns varying the diameters of the inhibitory zone.

The concentration of 500µg/ml of nanoparticles showed a higher inhibition zone than AK antibiotic on 4 isolates (2 clinical including *Staphylococcus aureus 16, Escherichia coli 4* and 2 environmental isolation *Staphylococcus aureus 1, Staphylococcus aureus 3*). In contrast, the antibiotic LEV has the highest inhibitory effect against all bacterial isolates. Also, the concentration 1000 µg/ml showed a higher impact than AK on two bacteria (1 clinical *Aeromonas spp.* and 1 environmental *Escherichia coli 3* isolates). Diagrams (1,2,3 and 4)illustrate the antibacterial activity of different TiO_2 NPs concentrations against studied bacteria.

3Study the synergistic activity between antibiotics and TiO2 nanoparticles

500 and 1000µg/ml TiO_2 nanoparticles concentrations were mixed with each antibiotic AK and LEV and studied against bacteria.AK plus the concentration 500µg/ml TiO2 showed an increased inhibitory zone against 7 isolates (3 clinical *Staphylococcus aureus* 16, *Enterobacter cloacae, Pseudomonas aeruginosa*,4 environmental *Staphylococcus aureus 1, Staphylococcus aureus 2, Staphylococcus aureus 3, Escherichia coli 1*) on mixing the concentration 1000µg/ml TiO_2 nanoparticles with AK antibiotic shows causes an increase in the inhibition zone against(*Staphylococcus aureus 1, Staphylococcus aureus 2, Staphylococcus aureus 3, Escherichia coli 1, Pseudomonas aeruginosa 3*, where is the other bacterial response to AK.AK mixed with 500 and AK with 1000 µg/ml causes no significant difference.

LEV antibiotic showed no difference in its effect on (7 clinical *Aeromonas spp., Staphylococcus aureus 16, Klebsiella oxytoca, Enterobacter cloacae, Burkholderia cepacia, Escherichia coli 4, Escherichia coli 3* and 3 environmental *Staphylococcus aureus 1, Staphylococcus aureus 2, Staphylococcus aureus 3*), while LEV mixed with 500 µg/ml causes increased inhibitory zones on (*Burkholderia cepacia, Escherichia coli 3, Staphylococcus aureus2, and Pseudomonas aeruginosa 3*) , and LEV mixed with 1000µg/ml increased the effect antibiotic effect on (2 clinical *Burkholderia cepacia, Pseudomonas aeruginosa* and 1 environmental *Pseudomonas aeruginosa3*) and the diagrams (3,4) illustrate the synergistic effect on TiO_2 NPs and the chosen concentration of antibiotics.

Fig: (1 and 2) showed the antagonistic effect of AK, LEV and the synergistic effect with concentrations of TiO_2 NPs on the *Staphylococcus aureus* and *Klebsiella oxytoca* bacteria as showed in table (3).

No.	Bacterial	Tio2 NP					AK 10 mcg	LEV 5 mcg
		20µg\ml	50µg\ml	100 µg\ml	500 µg\ml	1000 µg\ml		
Clinical isolates								
1-	Aeromonas spp.	--	--	--	30	40	R	22
2-	*Staphylococcus aureus* 16	--	--	--	--	--	25	41
3-	*Klebsiella Oxytoca*	--	--	--	25	18	17	38
4-	*Enterobacter cloacae*	--	--	--	--	37	17	40
5-	*Burkholderia cepacia*	--	--	--	--	25	10	30
6-	*Escherichia coli 4*	--	--	--	--	19	20	40
7-	*Escherichia coli 3*	--	--	--	--	19	17	30
8-	*Pseudomonas aeruginosa*	--	--	--	--	--	16	35
9-	*Pseudomonas aeruginosa 2*	--	--	--	--	--	18	38
10	*Pseudomonas aeruginosa1*	--	--	--	17	--	19	38
Environmental isolates								
11	*Staphylococcus aureus* 1	non	non	non	20	13	40	40
12-	*Staphylococcus aureus* 2	--	--	--	--	--	10	30
13-	*Staphylococcus aureus* 3	--	--	--	20	11	30	40
14-	*Escherichia coli 1*	--	--	--	13	22	17	30
15-	*Pseudomonas aeruginosa 3*	--	--	--	18	--	17	30

Table 2. The effectiveness of different concentrations of TiO_2 NP compared with the antibiotics LEV, AK.

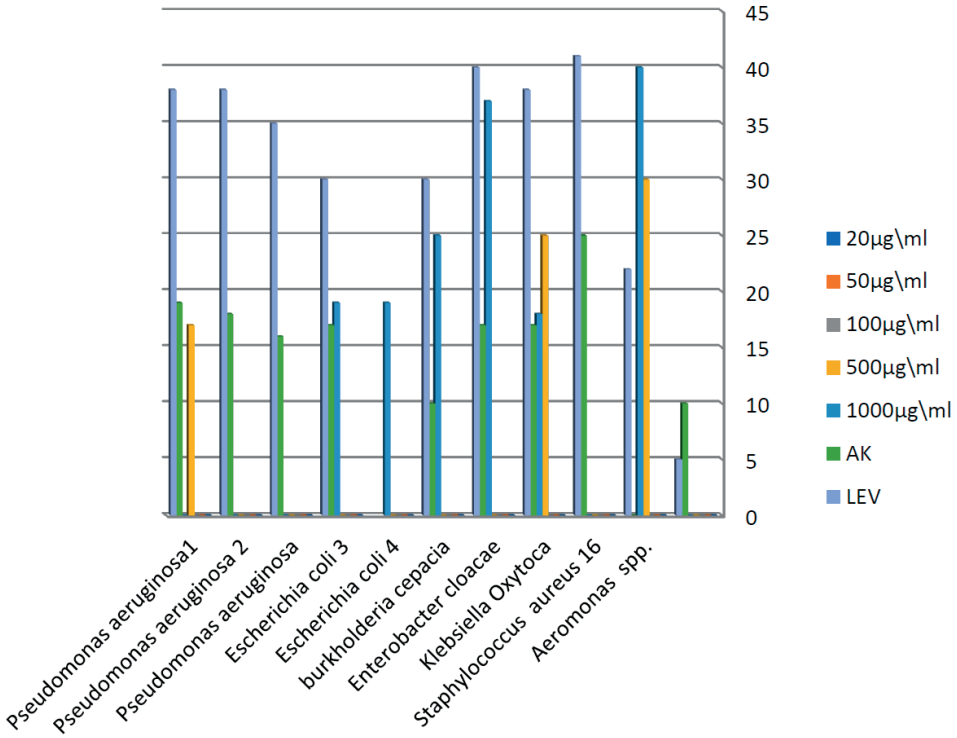

Figure 2. The effect of different concentrations of TIO_2 NPs on Clinical bacteria.

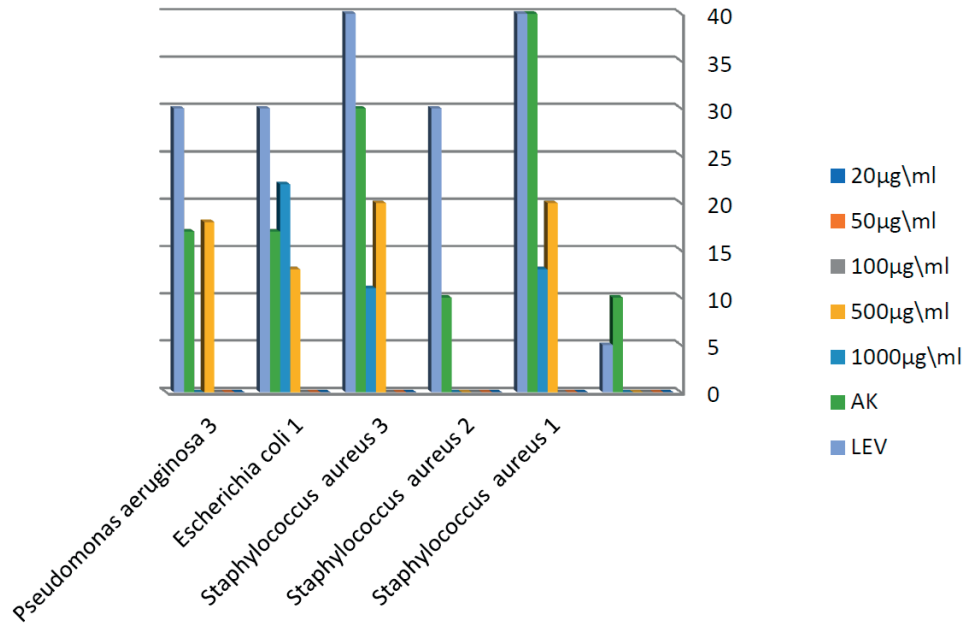

Figure 3. The effect of different concentrations of TiO_2 NPs on environmental bacteria.

Discussion

Research has shown that the field of application of NPs in the medical field can increase the performance and effectiveness of therapeutic drugs, and mixing drugs with NPs leads to a process of accumulation in diseased tissues of the mixed substance medicine[23].

Titanium dioxide (TiO_2) inorganic nanoparticles have been used in the last decades because they may have a general mechanism of toxicity against bacteria[9,24].

According to several studies, it is believed that the metal oxides carry the positive charge while the microorganisms have negative charges; this causes electromagnetic attraction between microorganisms and the metal oxides, which leads to oxidization and, finally, death of microorganisms[25]. They cause pits or holes, on bacterial cell wall could be associated with internalized particles, leading to increased permeability and cell death[26,27] ((In other words, the difference in electrical charges between polluted metals and living organisms causes a state of attraction between them, which leads to the accumulation of heavy metals in the body of the living organism and it dies.))TiO_2 nanoparticles due to their small size and high surface to volume ratio undergo a higher level of interaction with the bacterial cells surface than the larger particles, resulting in a high antibacterial activity[28].

The result of this study may differ from other studies

No.	Bacteria	AK	AK+Ttio2 500µg\ml	AK+ Tio2 1000	LEV	LEV+Tio2 500µg\ml	LEV+Tio2 1000 µg\ml
Clinical isolates							
1-	Aeromonas spp.	20	20	20	40	40	40
2-	*Staphylococcus aureus* 16	24	28	25	40	40	40
3-	*Klebsiella Oxytoca*	20	20	20	40	40	40
4-	*Enterobacter cloacae*	18	24	16	40	40	40
5-	*burkholderia cepacia*	20	20	20	40	48	44
6-	*Escherichia coli 4*	20	20	20	40	40	40
7-	*Escherichia coli 3*	22	22	20	40	50	40
8-	*Pseudomonas aeruginosa*	30	32	30	48	48	48
9-	*Pseudomonas aeruginosa 2*	--	--	--	--	--	--
10-	*Pseudomonas aeruginosa1*	---	17	------	----	38	19
Environmental isolates							
11-	*Staphylococcus aureus* 1	26	28	26	40	40	40
12-	*Staphylococcus aureus* 2	30	32	34	40	42	40
13-	*Staphylococcus aureus* 3	24	26	26	40	40	40
14-	*Escherichia coli 1*	22	24	22	S	S	S
15-	*Pseudomonas aeruginosa 3*	20	20	20	36	50	40

Table 3. Synergistic result between TIO_2 and the antibiotics (LEV, AK).

in the response of bacterial species towards TiO_2 NPs at different concentrations because there is no photocatalytic or UV activation; also, the study was done by using the disk diffusion method, whereas many studies done in a liquid medium which may be favoring the close interaction between the suspended nanoparticles and the Gram-positive microbial cells, which could better attach and anchor to the surface of the microbial cells, causing structural changes and damages leading to cell death[29].

TiO_2 NPs can directly oxidize components of cell signaling pathways and even change gene expression by interfering with transcription factors. This result suggested that TiO_2 NPs affect the microorganisms by not only oxidative damage but also bacteria aggregation and biofilm formation, which directly influenced pathogenicity[30].

Conclusions

Antibiotic treatment alone (or sometimes) fails to eradicate microbial infections like a medical device-related biofilm. Combining TiO2 NPs promising agent with antibiotics may be possible to eliminate s in the future.

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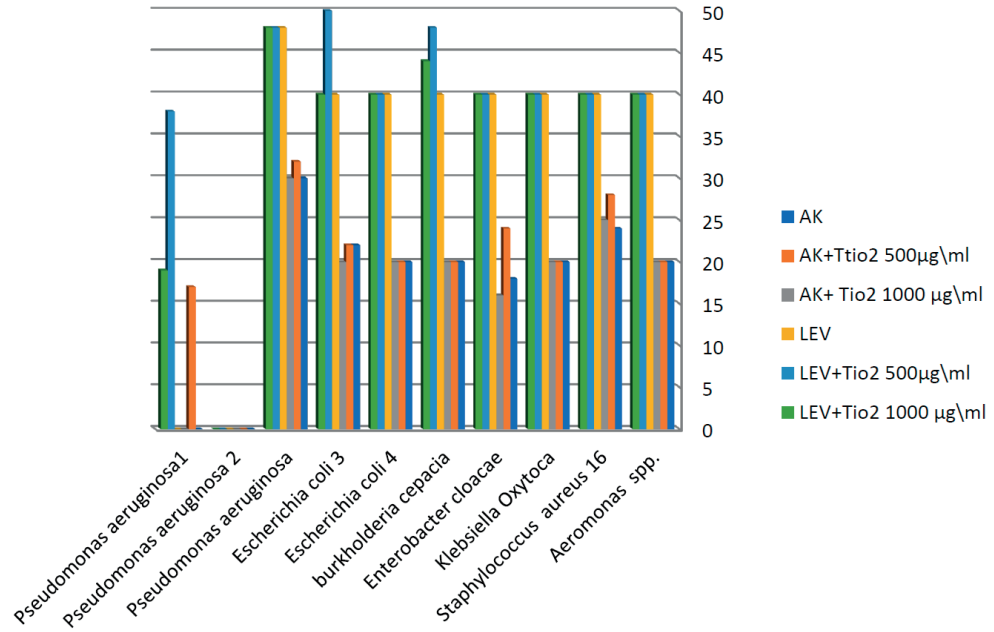


Figure 4. The synergistic effect of at TiO_2 NPs a chosen concentration with of antibiotics against clinical bacterial isolates.

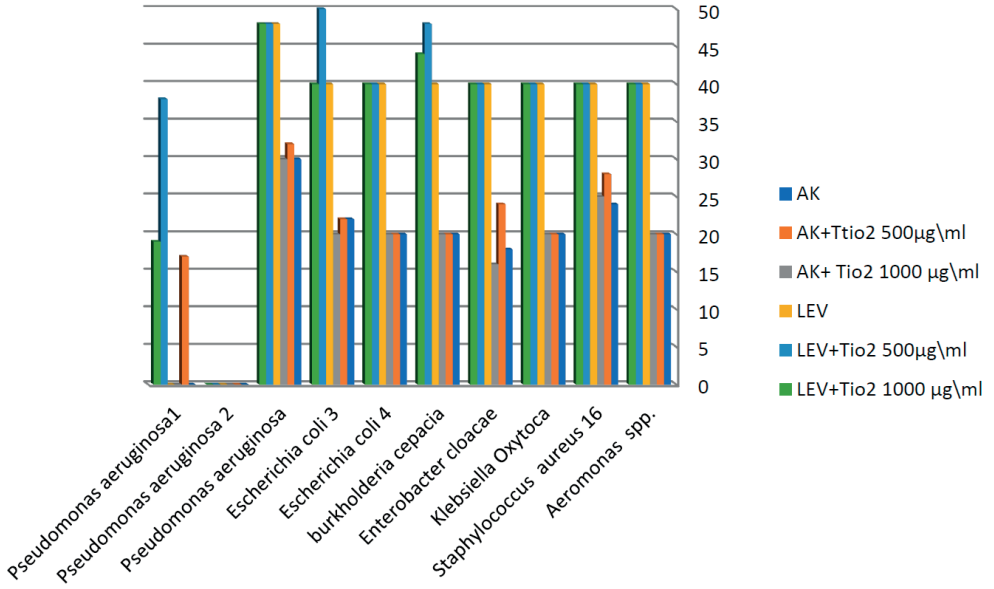
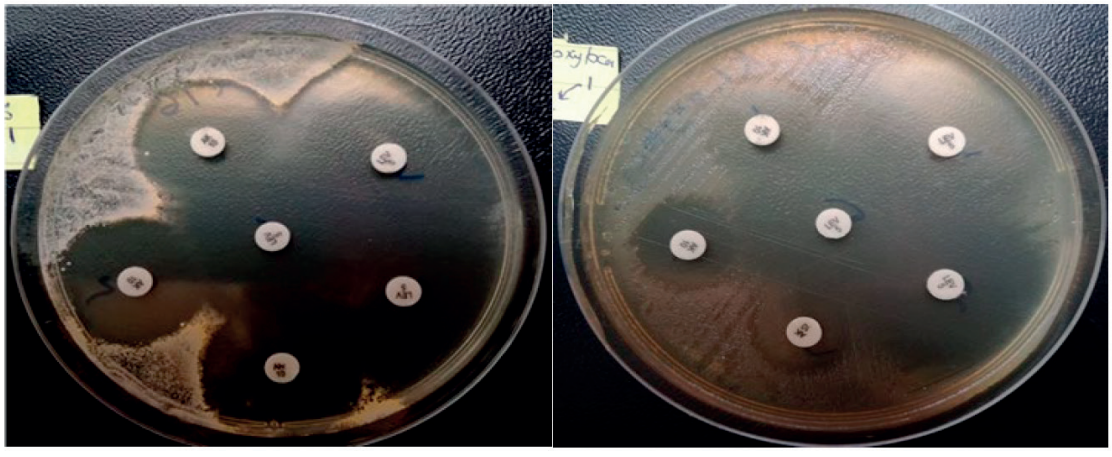


Figure 5. The synergistic effect of TiO_2 NPs at a chosen concentration with antibiotics against environmental bacteria.



(a) (b)

Figure 6. The inhibitory activity of the (Tio_2).

Conflicts of Interest

The authors declare that they have no conflict of interest in this study.

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