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ORIGINAL RESEARCH

# Comparative Study of Antibacterial Effects of Titanium Dioxide Nanoparticles Alone and in Combination with Antibiotics on MDR *Pseudomonas aeruginosa* Strains

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**Introduction:** The efficacy of several antimicrobial agents has been hindered due to the increasing frequency of multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains. So, the need for new antibacterial drugs or drug combinations is urgent. Recently, desirable antibacterial effects were reported for many metals nanoparticles such as $TiO_2$ nanoparticles (TDNs).

**Purpose:** This study aims to investigate the prevalence of MDR *P. aeruginosa* and assess the efficiency of TDN in the treatment of MDR *P. aeruginosa*-associated infections.

**Materials and Methods:** The synthesis of TDN by the sol-gel method was carried out. Particle size measurements and morphology were done using dynamic light scattering (DLS) and high-resolution transmission electron microscopy (HR-TEM). To investigate the physical and chemical changes of drugs due to the combination, the tested drugs, both alone and in combination with TDN, were subjected to differential scanning calorimetry (DSC), infrared (IR) spectroscopy, and X-ray diffraction studies. Antimicrobial susceptibility was detected by agar disc-diffusion assay. The minimum inhibitory concentration (MIC) of TDN and the tested antibiotics were assessed by the agar dilution method. Checkerboard analysis was performed to determine the combined effect of TDN and the tested antibiotics against 25 MDR *P. aeruginosa* strains.

**Results:** TDNs were prepared with an average particle size of $64.77 \pm 0.14$ nm with an accepted polydispersity index (PDI) value of $0.274 \pm 0.004$. TEM showed that the particles were shaped into irregular spheres. Twenty-five *P. aeruginosa* isolates that were absolutely resistant to cefepime (100%), highly resistant to ceftriaxone (96%), amikacin (80%), and ciprofloxacin (76%) were selected. Superior antibacterial activity of TDN was observed against the selected 25 MDR *P. aeruginosa* isolates. The combination of TDN and cefepime were found to show synergistic activity against all tested isolates followed by ceftriaxone (96%), amikacin (88%), and ciprofloxacin (80%).

**Conclusion:** Using TDN in combination with antibiotics can help in the treatment of MDR *P. aeruginosa*-associated infections. So, preparation of topical pharmaceutical dosage forms containing a combination of these antibiotics and TDN can be useful against MDR *P. aeruginosa*.

**Keywords:** MDR *P. aeruginosa*, titanium dioxide nanoparticle, checkerboard assay

## Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen that causes a variety of diseases ranging from mild to severe life-threatening infections and considered as one of the main causes of nosocomial or hospital-acquired infections.[1–3] *P. aeruginosa* infections

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are difficult to eradicate by many antimicrobial agents due to its intrinsic resistance (low outer membrane permeability, efflux mechanisms, and inactivity of the antibiotics by enzymes), acquired resistance (mutations in genes targeted by the antibiotics), adaptive resistance (biofilm formation),[4] and various virulence factors (extracellular proteases, toxins).[5] Treatment of these infections has become a great challenge because of the ability of this bacterium to resist a variety of antibiotics, including aminoglycosides, quinolones, and β-lactams. So, combination therapy may help in the reduction of the mortality rate of patients with severe *P. aeruginosa* infections.[4]

The emergence of multidrug-resistant (MDR), Extensive Drug-Resistant (XDR), and pandrug-resistant bacteria increased the need for the development of alternative strategies to treat bacterial diseases. One of these strategies is the use of nanoscale materials.[6] The nanoparticles used as antimicrobial agents due to its ability to penetrate bacterial membranes, disrupt biofilm formation, and be good carriers of antibiotics.[4] Metallic nanoparticles are attracting a great deal of attention because of their selectivity and potential success in the biological and pharmaceutical applications.[7,8] Many metal oxides nanoparticles showed good antimicrobial activity such as the following: $TiO_2$, ZnO, MgO, CuO, SiO2, and CoO. They are less toxic and heat resistant and exhibit marked effectiveness against resistant strains of some microorganisms. Besides, they may act as mineral elements supplement that is essential to human cells.[9] Titanium oxide nanoparticles showed good inhibitory activity against bacterial growth due to its small nanometer scale and potent oxidizing power.[3] This study was conducted to determine the prevalence of MDR *P. aeruginosa* that causes many diseases and to assess the efficiency of TDN alone and in combination with different antibiotics in the treatment of MDR *P. aeruginosa*.

## Materials and Methods
### Reagents
Titanium Tetrachloride ($TiCl_4$, 99.5%) was obtained from Loba chemie, India.

Ceftriaxone sodium, amikacin sulfate, cefepime hydrochloride, and ciprofloxacin were obtained from Pharco B company, Egypt.

## Synthesis of $TiO_2$ Nanoparticles (TDN)
TDN was prepared by the sol-gel method. The procedures were carried out according to the previously described method with slight modifications.[10] Briefly, 5 mL of titanium tetrachloride were added to 50 mL of absolute Ethanol; the mixture was stirred for 30 min using a magnetic stirrer till a yellow sol phase was formed. While stirring, 200 mL of distilled water were added until the solution became colorless and clear. The solution was further stirred for 45 min while the temperature was kept at 25–30°C. The formed TDN was collected by centrifugation at 10,000 rpm for 15 min, washed by distilled water several times, and then dried at 50°C for 30 h.

## Characterizations of $TiO_2$ Nanoparticles (TDN)
### Dynamic Light Scattering (DLS) Analysis and Zeta Potential Measurement
A liquid sample was diluted several times using Milli-Q water purifier and analyzed using DLS (Zetasizer Nano-ZS instrument, Worcestershire, United Kingdom). By placing samples in the cuvette, both particle sizes and the zeta potentials of the synthesized nanoparticles were determined. The samples were prepared for analysis at room temperature (25°C) and measured at 37°C in triplicates. Results were presented as mean ± SD (standard deviation).

### Transmission Electron Microscopy
The morphological characteristics of TDN were investigated by a transmission electron microscope (TEM, Model 100 CX II, Tokyo, Japan).

## Compatibility Study
### Preparation of Physical Mixtures (PM)
Physical mixtures of each of the tested drugs with TDN at weight ratio 1:1 were prepared by gentle mixing in a mortar using a pestle.

### Differential Scanning Calorimetry (DSC) Study
The DSC patterns were obtained by heating the samples from 30–600°C at a scanning rate of 10°C/min under a stream of nitrogen gas at a flow rate of 40 mL/min using SDT (simultaneous DSC\TGA) Q600, USA. Samples of 4 mg were accurately weighed and encapsulated into flat-bottomed aluminum pans with crimped-on lids. The procedures involved heating of the sample contained in the aluminum pan and a similar empty reference pan at the predetermined heating rate. The differential heat flow between the sample and the reference was recorded and presented graphically.

### Infrared (IR) Spectroscopy Study

IR spectroscopy was used to detect chemical interactions between TDN and the tested drugs using FTIR Perkin Elmer Spectrum One, UK. It was carried out using the potassium bromide disk method. Samples, 1–2 mg each, were mixed with potassium bromide, compressed at a pressure of 6 ton/cm$^2$ into discs, and scanned over the range of 400–4000 cm-$^1$ using a blank pellet of potassium bromide as a reference.

### X-Ray Diffraction Study

Powdered samples of TDN, tested drugs, and physical mixtures were analyzed using X-ray. The X-ray diffractograms were obtained using Panalytical X'PERT PRO, Holland. A single-crystal graphite monochromator was employed. The target was CuK∝ radiation, operating at a current of 40 mA and 40 kV. Diffractograms were obtained using continuous scan mode with 2 θ values ranging from 5 to 80 degrees at a rate of 2 degrees/minute.

## Isolation and Identification *of Pseudomonas aeruginosa* Isolates

One hundred clinical specimens were examined for *P. aeruginosa* (50 urine and 30 wound exudate specimens and, five sputa, ear discharge, burn swab, and eye discharge specimens). All specimens were collected from different hospitals in Minia governorate, Egypt. All specimens were examined for the presence of *P. aeruginosa* by the conventional microbiological procedures[11] and confirmed by biochemical reactions.

## Antimicrobial Susceptibility Testing

*Pseudomonas aeruginosa* isolates underwent antimicrobial susceptibility test in six antibiotic discs using the Kirby-Bauer disc diffusion method.[12] The antibiotics tested were ciprofloxacin (CIP, 5 μg), amikacin (AK, 30 μg), ceftriaxone (CRO, 30 μg), levofloxacin (LEV,10 μg), imipenem (IPM, 10 μg), and cefepime (FEP, 30 μg) discs (Oxoid, England).

## Determination of Minimum Inhibitory Concentrations (MICs) of the Tested Antibiotics and TND

Minimum Inhibitory Concentrations (MIC) were expressed as the lowest antibacterial agent concentrations that caused a total inhibition of bacterial growth for 24 hours. The MICs of cefepime, ceftriaxone, amikacin, ciprofloxacin, and TDN were determined for 25 MDR *P. aeruginosa* isolates using the agar dilution method. Overnight cultures of the tested isolates in a Mueller-Hinton Broth (MHB) were adjusted to a cell density of 10$^7$ CFU/mL. Then, the bacterial culture spots were applied to the surface of a dry Muller-Hinton Agar (MHA) containing the tested antibiotics and TDN of concentrations ranged from 1 to 1024 μg/mL using a multi-inoculator. Plates were incubated at 37 °C for 18–24 h and examined for growth. Spots showing no growth were defined as MIC.[13]

## Determination of the Synergistic Effect of TDN with Antibiotics by Checkerboard Assay

The effect of combinations of TDN with the tested antibiotics at sub-MIC values was examined by checkerboard titration tests. The MICs of antibiotics and TDN in combination were determined by the agar dilution method against 25 MDR strains, and the fractional inhibitory concentration index (FICI) was determined as described before.[14]

## Results and Discussion
### Characterizations of TDN

In this study, TDN was prepared using the sol-gel method. The average particle size determined by DLS was $64.77 \pm 0.14$ nm. This small size of nanoparticles provides a large surface area that enhances the interactions with the microbes and increases the range of antimicrobial activities.[15] The poly dispersive index (PDI) was $0.274 \pm 0.004$, which is lower than 0.3, indicating the homogenous population of the particle size[16] as shown in Figure 1. According to literature, this value suggests the stability of the prepared TDN with storage as zeta potential value was 23.8 mV. As TDN suspension has large positive or negative zeta potential, particles in suspension will tend to repel from each other which prevents aggregation and agglomeration of the particles.[17] The morphology was investigated by a high-resolution transmission electron microscope (Figure 2). Images showed that the prepared nanoparticles were nearly spherical in shape of irregular edges with little aggregations at different magnifications (Figure 2A, C, and D). Particle size histogram showed that the maximum distribution of particle sizes was in the range of 60–80 nm (Figure 2B), and that agrees with DLS results. Many nanoparticle shapes with nonuniform size and superficial agglomeration were reported for TiO$_2$, which appeared to be a normal reaction during the sol-gel method synthesis.[18,19]

### Diffraction Scanning Calorimetry Studies

DSC is a significant tool to predict the physicochemical changes associated with drug interactions of the tested
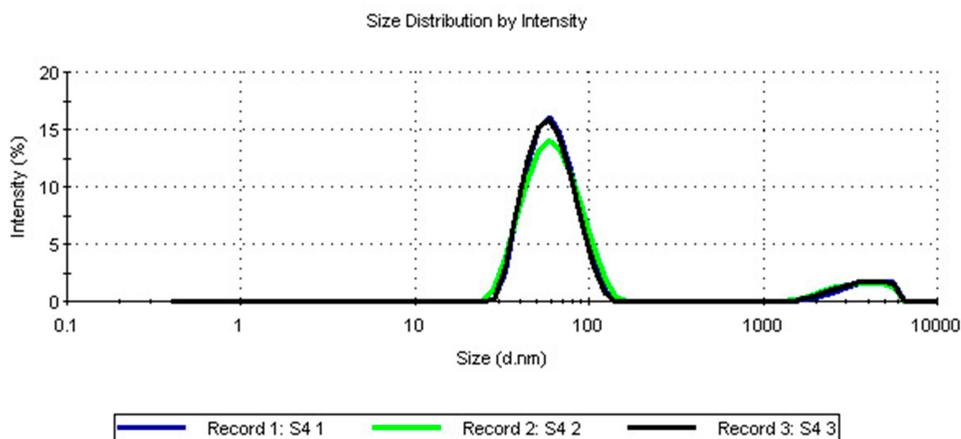
**Figure 1** Particle size distribution of the formed titanium dioxide nanoparticles measured by dynamic light scattering analysis.
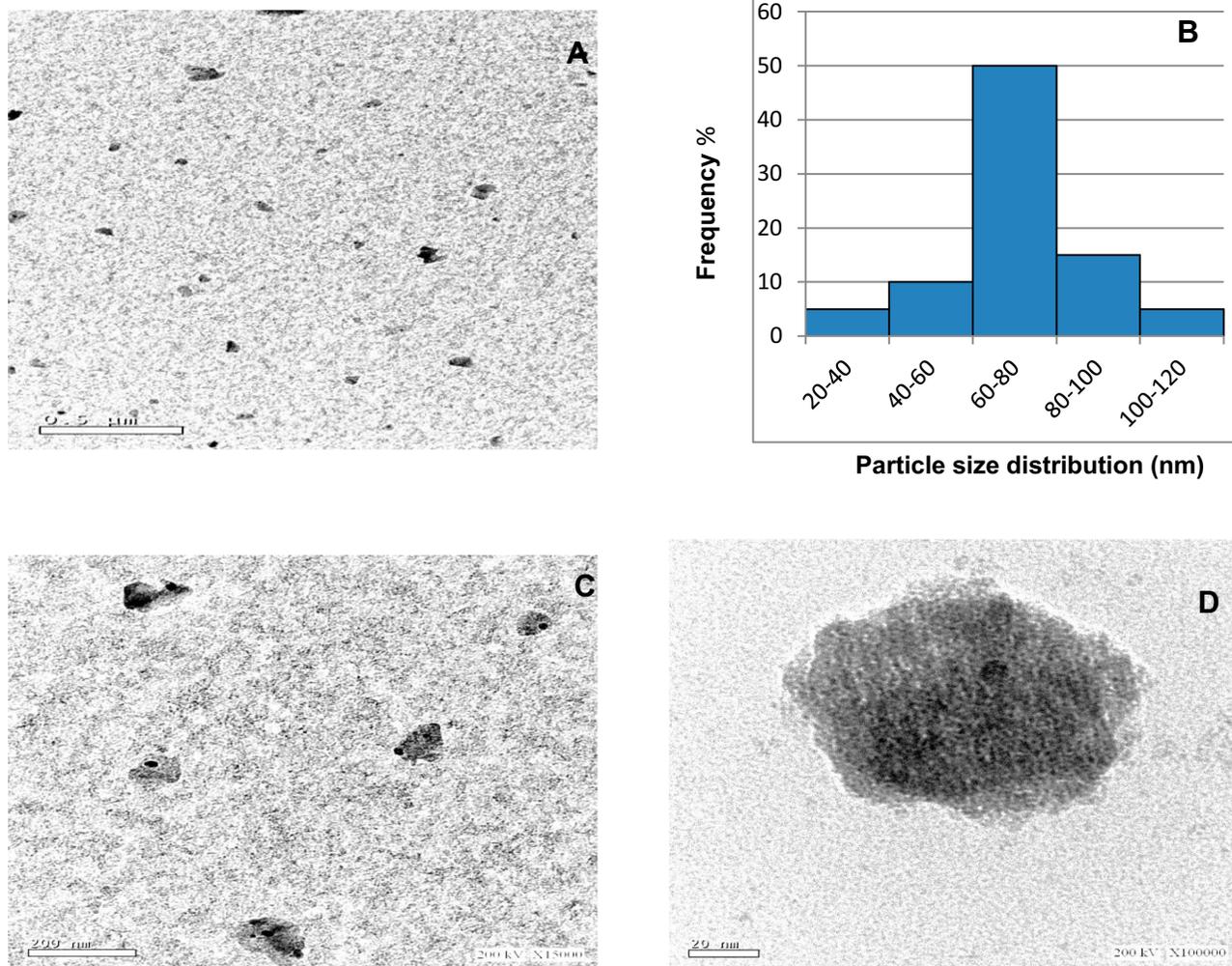


**Figure 2** High-resolution transmission electron microscopy images at different magnifications (**A**: ×5000, **C**: ×15,000 **D**: ×100,000) showing the prepared titanium dioxide nanoparticles as spherical shape with an irregular edges. (**B**) Particle size distribution of it.

antibacterial agents and TDN. Drug interactions may be detected through shifts in peaks onset, area, or even disappearance of peaks. Sometimes the appearance of new peaks can be considered evidence of an interaction. All tested drugs and TDN showed dehydration peaks at different temperatures that appeared also in their

corresponding Physical Mixtures (PM) (usually below 100°C) as shown in Figure 3.

The DSC thermogram of TDN showed a broad endothermic peak around $80°C$ to$120°C$ due to dehydration. Besides, a second small broad endothermic peak appeared on the curve at $330°C$ which may have occurred due to the crystallization of the amorphous $TiO_2$.[20] Concerning the amikacin system, the drug showed its melting point endothermic peak at $256°C$, per other reports mentioned elsewhere.[21] There were no significant changes

in the Physical Mixture (PM) suggesting the absence of a physicochemical interaction between the drug and the prepared TDN (Figure 3A). For the ciprofloxacin system, the drug showed its melting point endothermic peak at its proper position at $315°C$, very close to the reported value-[22–24] without any considerable changes in peak position in Figure 3B.

Figure 3C shows the DSC thermograms of the ceftriaxone system. The drug produced its melting point endothermic peak at $181°C$,[25] identical to the DSC
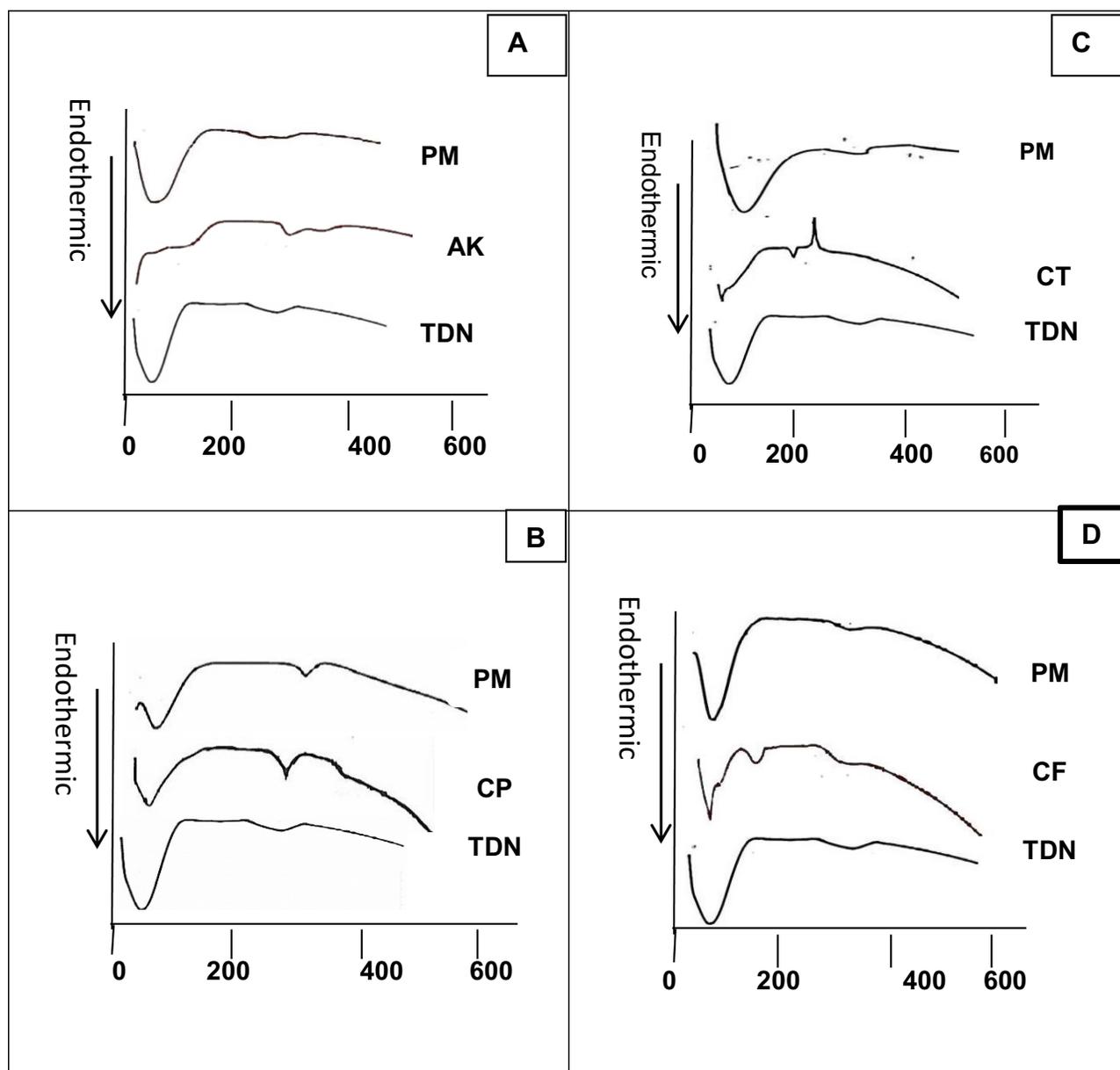


**Figure 3** Diffraction scanning calorimetry analysis of (**A**) TDN, amikacin and its physical mixture; (**B**) TDN, ciprofloxacin and its physical mixture; (**C**) TDN, ceftriaxone and its physical mixture; (**D**) TDN, cefpime and its physical mixture.
**Abbreviations:** TDN, titanium dioxide nanoparticles; AK, amikacin; CP, ciprofloxacin; CT, ceftriaxone; CF, cefpime; PM, physical mixture.

thermogram of the PM. Cefepime produced its characteristic peak at 185°C which is corresponding to its melting point. The peak appeared nearly in the same position in PM without any considerable changes[26] (Figure 3D).

## Infrared (IR) Spectroscopy Studies

FTIR was applied to assess the chemical composition and quality of the synthesized TDN as shown in Figure 4. FTIR spectrum of the synthesized TDN showed a small band in 1624 cm$^{-1}$ which is characteristic to o-Ti-o bond,
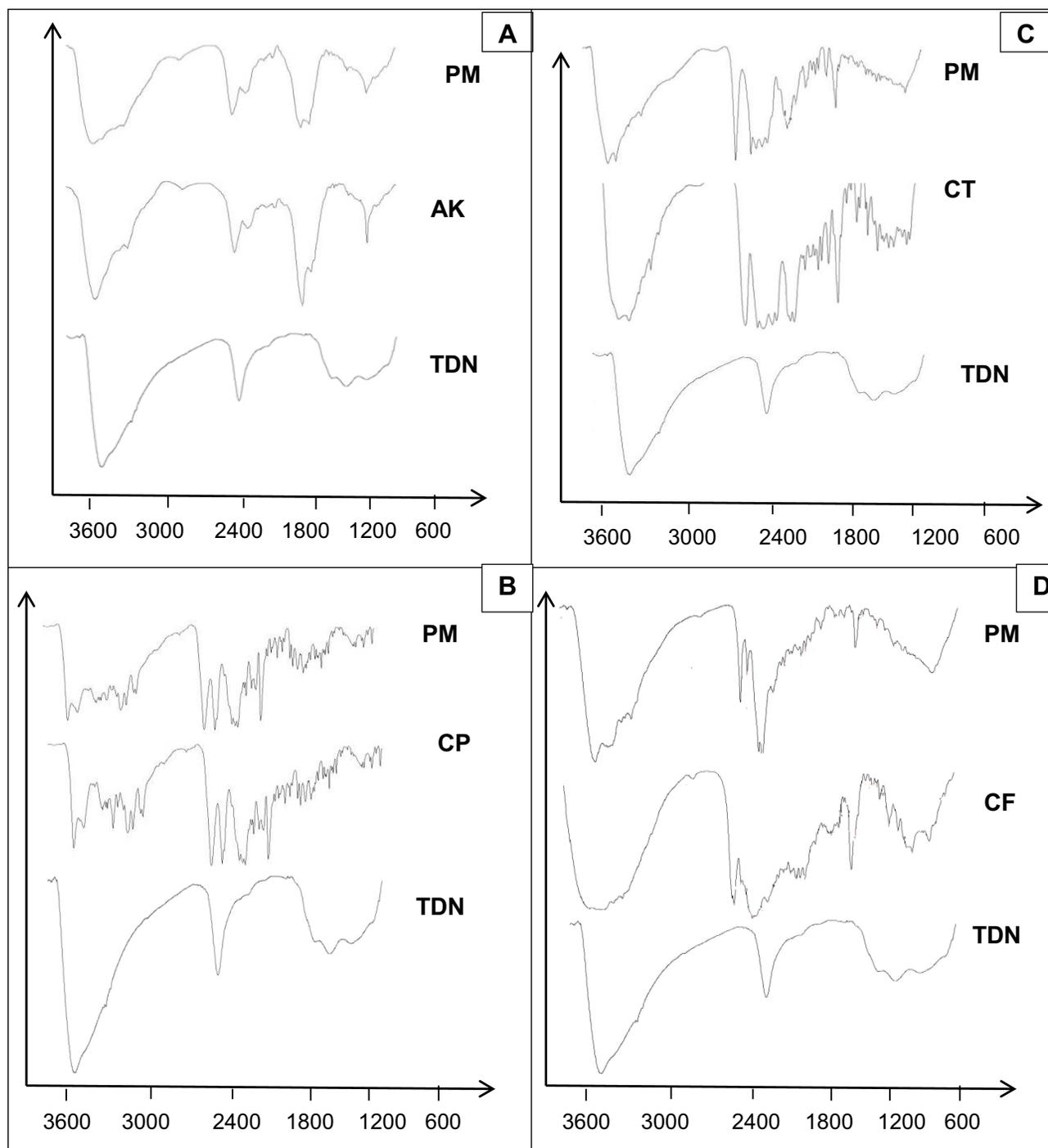


**Figure 4** Fourier transform infrared spectra of (**A**) TDN, amikacin and its physical mixture; (**B**) TDN, ciprofloxacin and its physical mixture; (**C**) TDN, ceftriaxone and its physical mixture; (**D**) TDN, cefpime and its physical mixture.
**Abbreviations:** TDN, titanium dioxide nanoparticles; AK, amikacin; CP, ciprofloxacin; CT, ceftriaxone; CF, cefpime; PM, physical mixture.

a broadband at 3385 cm$^{-1}$, which is related to O-H stretching in agreement with previous studies[27,28] that confirm the formation of TDN. To estimate the fundamental interactions of TDN and the tested drugs, Physical Mixtures (PM) were examined employing the FTIR spectrophotometer for detecting typical absorption bands. Figure 4A shows the broadband at 1638 cm$^{-1}$ corresponding to N–H bending vibration of primary aromatic amines of amikacin sulfate. This band appeared in PM in the same position, suggesting the absence of interaction between drugs and TDN.[29]

For ciprofloxacin, the major character bands in the IR spectrum included sharp and small bands at 1624 cm$^{-1}$ and 1668 cm$^{-1}$ respectively (corresponding to the vibration absorption of CH$_2$ on benzene ring), a sharp band at 1710 cm$^{-1}$ (due to stretching vibration attributed to a carbonyl group), and small bands at 1399 and 944 cm$^{-1}$ (corresponding to O-H).[30] Nearly all the previous bands appeared in the PM (Figure 4B). The FT-IR spectrum of ceftriaxone (Figure 4C) showed characteristic bands at 3429 cm$^{-1}$ (N–H) stretching of the H-bonded amide group, 1742 cm$^{-1}$ β-lactam C=O stretching vibrations, and 1610 cm$^{-1}$ oxime C=N stretching vibrations. All bands appeared very close to the reported data.[25] Characteristic bands of ceftriaxone appeared in the same position in the PM. Cefepime showed main characteristic bands at 2935 cm$^{-1}$, 1773.29 cm$^{-1}$, and 1655 cm$^{-1}$ corresponding to O-H stretching, β-lactam C=O stretching, and amide C=O, respectively. That is close to the reported value.[26] Bands appeared in the PM in their proper positions without any significant shift (Figure 4D). Data of IR and DSC supported the absence of interaction between tested drugs and TDN.

## X-Ray Diffraction Studies

The X-ray diffractogram of TDN, the tested drugs, and their combinations in the PM are shown in Figure 5. TDN showed less crystalline or amorphous structure which is somewhat similar to a brookite polymorph. Growth of brookite under acidic conditions has been consistently reported when the water-soluble Ti complexes are replaced by TiCl$_4$. Also, for most of the reported work, it was noticed that the use of a water-based growth environment has been a common denominator on the growth of brookite nanostructures.[31] Regarding amikacin, the X-ray diffractogram revealed a crystalline structure of the drug with major diffraction peaks at 2 θ values of 17.57°, 18.08°, and 33.84°. The drug retained its crystalline structure in the PM without detectable changes in its characteristic peaks (Figure 5A). The same results were observed in ciprofloxacin PM. The major diffraction peaks at 2 θ values of the drug appeared at 19.18°, 26.4°, 18.8°, 29°, 25.66°, 23°, and 19.7° without significant changes (Figure 5B). Concerning ceftriaxone, the X-ray diffractogram showed major diffraction peaks at 2 θ values 21.09°, 22.66°, 23.67°, 18.80°, and 18.28° which revealed the high crystallinity of the drug.

The diffractogram of the PM with TDN showed that all characteristic peaks of the drug appeared at the same two theta values without any considerable changes (Figure 5C). Concerning cefepime, the X-ray diffractogram of the drug revealed a high degree of crystallinity with major diffraction peaks at 2 θ values of 27.72°, 23.18°, 19.37°, 16.7°, and 22.63°. No detectable changes were observed in the position of peaks in the PM (Figure 5D).

From the previous data, it could be concluded that the four above mentioned drugs kept their crystalline structure in the PM with TND, which confirms the DSC and FTIR. The physicochemical compatibility between the investigated drugs and the prepared TDN allows for the combination and preparation of pharmaceutical dosage forms containing each drug with nanoparticles to get the benefits of adding TDN to the antibiotics to overcome the big problem of MDR bacteria such as *P. aeruginosa*.

## Prevalence of *Pseudomonas aeruginosa* in Clinical Specimens

In this study, 67 *P. aeruginosa* strains were isolated from 100 different clinical specimens.

This result was similar to other results reported from Iraq (69%)[32] and lower than others.[13,33,34] Geographic climatic and hygienic factors may play important roles in the relative variability of the incidence rate. Prevalence of *Pseudomonas aeruginosa* isolates according to the type of clinical specimen were 39 (78%) from urine, 20 (66.7%) from wound exudate, 5 (100%) from ear discharge, 2 (40%) from eye discharge, and 1 (20%) from sputum specimens. *P. aeruginosa* had the highest incidence rate in ear infections (100%). No *P. aeruginosa* were isolated from burn specimens. Appiah-Korang et al (2014) showed that *Pseudomonas* species were the most common bacteriologic cause of ear discharge since it is a widespread
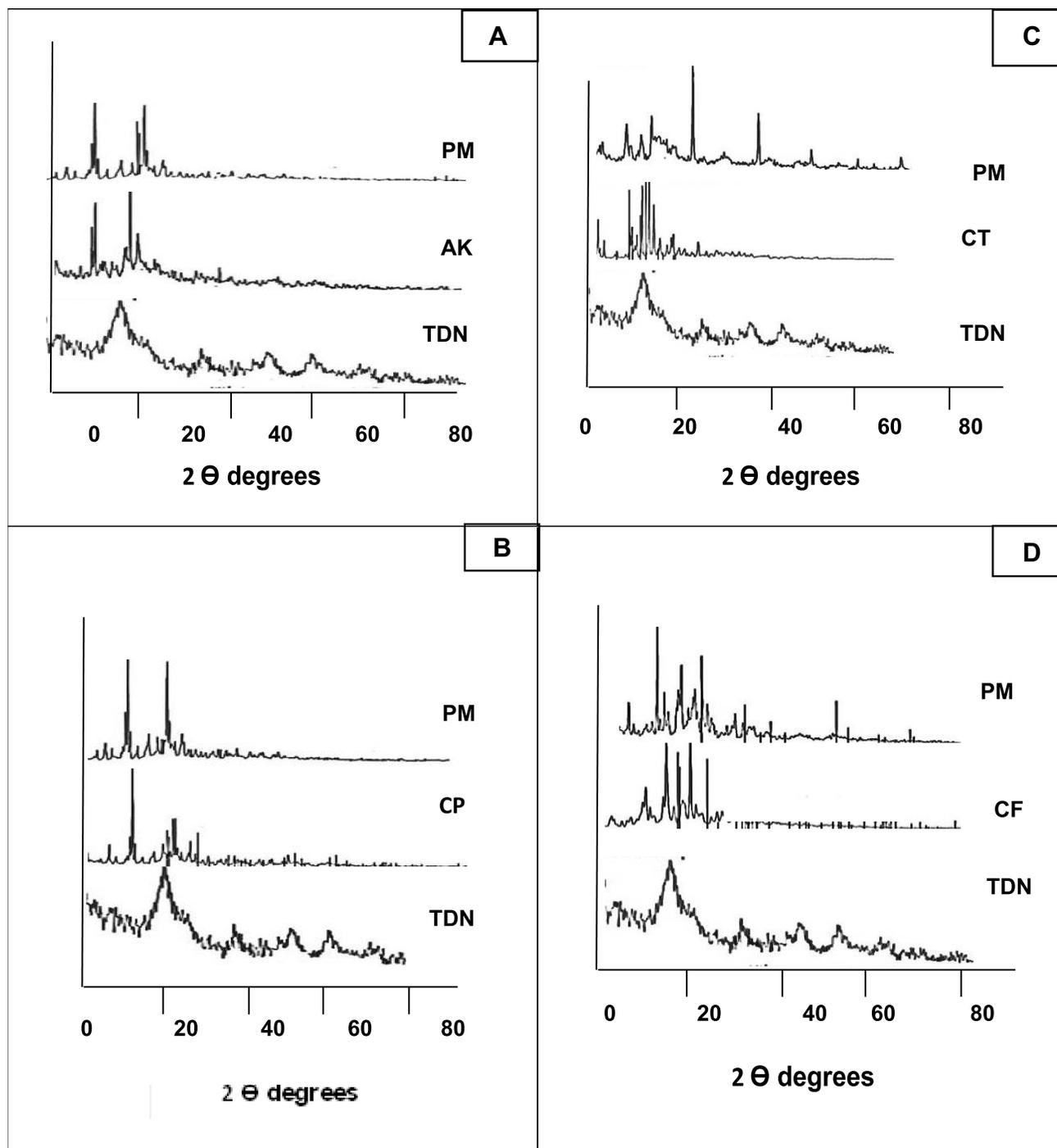
**Figure 5** X-ray diffraction pattern of (**A**) TDN, amikacin and its physical mixture; (**B**) TDN, ciprofloxacin and its physical mixture; (**C**) TDN, ceftriaxone and its physical mixture; (**D**) TDN, cefpime and its physical mixture.

**Abbreviations:** TDN, titanium dioxide nanoparticles; AK, amikacin; CP, ciprofloxacin; CT, ceftriaxone; CF, cefpime; PM, physical mixture.

environmental organism that is usually found in warm and humid environments and is known to colonize the external auditory channel which is consistent with our findings.[35] In contrast, Mahmoud et al[36] and Abed Al-Azzawi et al[32] found that *P. aeruginosa* was mostly isolated from burn specimens (32.3%, 65%, respectively).

## Antibiotic Susceptibility of *P. aeruginosa* Isolates

Recently, Egypt has been considered among the countries that suffer from high rates of antimicrobial resistance. The present study showed a high resistance for the tested antibiotics. Sixty strains (89.5%) were found to be resistant to

ceftriaxone, 40 (59.7%) to cefepime, 21 (31.34%) to amikacin, 20 (29.8%) to imipenem, 17 (25.4%) to ciprofloxacin, and 8 (12%) levofloxacin.

A similarly high rate of resistance has been reported in many previous studies in Egypt.[37–39]

The rate of resistance to the third and fourth generation cephalosporins indicates the limited treatment choices in the hospitals of our area and the wide misuse of bactericidal antibiotics in the treatment of any infection. This leads to the accumulation of antibiotic resistance and cross-resistance between antibiotics and the appearance of multidrug-resistant (MDR) forms of *P. aeruginosa*.[40,41] However, Diab et al (2013) reported the lowest resistant rate to ceftazidime (46%) in comparison to other used antibiotics.[42]

Minimum Inhibitory Concentrations (MICs) values of ceftriaxone, cefepime, amikacin, and ciprofloxacin were detected against selected 25 MDR *P. aeruginosa* strains.

The selected 25 *P. aeruginosa* strains showed high resistance for the tested antibiotics (resist to three or more antibiotics) with different antibiotic resistance patterns and clinical specimens (13 urine,9 wound exudate, and three ear discharge specimens) as shown in Table 1. *P. aeruginosa* strains isolated from eye discharge and sputum specimens (2 and 1, respectively) were more susceptible to the tested antibiotics.

Determined Minimum Inhibitory Concentrations MICs of the studied antibiotics were compared to breakpoint values reported by CLSI as the basis for calculating the response. Tables 1 and 2 represent MICs of different antibiotics and antibiotic susceptibilities of the 25 MDR P. *aeruginosa* isolates. Results showed that all 25 MDR *P. aeruginosa* isolates were resistant to cefepime (100%) with MICs ranging from 32 to 512μg /mL. Ninety-six percent of strains were resistant to ceftriaxone with MICs ranging from16 to >1024 μg/mL. Eighty percent were resistant to amikacin with MICs of 16 to >1024 μg/mL, and 76% were resistant to ciprofloxacin with MICs 4 to 32 μg/mL. Our results showed a spread of multidrug-resistant strains due to indiscriminate use of antibiotics, lack of awareness, patient noncompliance, and nonavailability of antimicrobial testing facilities.[43]

## Susceptibility of *P. aeruginosa* Isolates to TDN

Antimicrobial NPs offer many distinctive advantages in reducing acute toxicity, overcoming resistance and lowering cost when compared to conventional antibiotics. Titanium dioxide nanoparticles have received considerable

**Table 1** Minimum Inhibitory Concentrations of Titanium Dioxide Nanoparticles and Different Antibiotics Against 25 *Pseudomonas aeruginosa* Isolates

| No.** | Type* | CP | CF | CT | AK | TDN |
|---|---|---|---|---|---|---|
| 1 | W | 8 | 256 | 32 | 512 | 8 |
| 2 | W | 32 | 512 | 1024 | 512 | 64 |
| 3 | W | 32 | 32 | 512 | 128 | 64 |
| 4 | U | 32 | 512 | >1024 | >1024 | 64 |
| 5 | ER | 32 | 512 | >1024 | 128 | 64 |
| 6 | U | 8 | 256 | 64 | 512 | 64 |
| 7 | U | 4 | 256 | 128 | 512 | 64 |
| 8 | U | 4 | 128 | 256 | 64 | 16 |
| 9 | U | 32 | 64 | 256 | 64 | 64 |
| 10 | U | <1 | 512 | 256 | 16 | 64 |
| 11 | U | 8 | 256 | 128 | 512 | <1 |
| 12 | W | 16 | 256 | 64 | 128 | 64 |
| 13 | W | 32 | 512 | >1024 | 1024 | 16 |
| 14 | W | 4 | 512 | 256 | 128 | 16 |
| 15 | U | <1 | 64 | 256 | 16 | <1 |
| 16 | U | 8 | 256 | 32 | 512 | 16 |
| 17 | U | 8 | 256 | 256 | 512 | 64 |
| 18 | W | <1 | 64 | 16 | 512 | 16 |
| 19 | W | 4 | 32 | 32 | 32 | 32 |
| 20 | U | 4 | 32 | 32 | 16 | <1 |
| 21 | ER | 4 | 32 | 32 | 64 | 64 |
| 22 | W | 4 | 32 | 32 | 512 | 64 |
| 23 | U | <1 | 256 | 64 | 64 | 64 |
| 24 | U | <1 | 256 | 64 | 64 | 64 |
| 25 | ER | <1 | 64 | 256 | 16 | 16 |

**Notes:** Type*, type of clinical specimen of isolate; No.**, serial number of specimens.
**Abbreviations:** U, urine specimen; W, wound exudate; ER, ear discharge; CP, ciprofloxacin; CF, cefpime; CT, ceftriaxone; AK, amikacin; TDN, titanium dioxide nanoparticles.

**Table 2** Antibiotic Susceptibility of *Pseudomonas aeruginosa* Isolates

| Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | no** | %* | no** | %* | no** | %* |
| Ceftriaxone | 0 | 0 | 1 | 4 | 24 | 96 |
| Cefepime | 0 | 0 | 0 | 0 | 100 | 100 |
| Amikacin | 4 | 16 | 1 | 4 | 20 | 80 |
| Ciprofloxacin | 6 | 24 | 0 | 0 | 19 | 76 |

**Notes:** no**, number of *Pseudomonas aeruginosa* isolates; *Percentages were correlated with total number of the selected *Pseudomonas aeruginosa* isolates (25).

attention as effective antimicrobial agents. The antimicrobial activity of TDN alone was tested at different concentrations against 25 MDR *P. aeruginosa* strains. Depending on the isolates' sensitivity, the MICs of TDN were ranged from 8 to 64 μg/mL. Where three strains (12%) had MIC less than 1 μg/mL (Table 1).

**Table 3** Checkerboard Results of Combination of Titanium Dioxide Nanoparticles and Antibiotics to 25 MDR *Pseudomonas aeruginosa* Isolates

| Drugs | Synergistic | | Additive | | ND | |
|---|---|---|---|---|---|---|
| | no** | %* | no** | %* | no** | %* |
| AK\TDN | 22 | 88% | 3 | 12% | 0 | 0 |
| CP\TDN | 20 | 80% | 3 | 12% | 2 | 8% |
| CT\TDN | 24 | 96% | 1 | 4% | 0 | 0 |
| CF\TDN | 25 | 100% | 0 | 0 | 0 | 0 |

**Notes:** *Percentages were correlated with number of MDR *Pseudomonas aeruginosa* isolates (N= 25); AK\TDN, combination of amikacin and titanium dioxide nanoparticles; no**, number of *Pseudomonas aeruginosa* isolates.
**Abbreviations:** MDR, multidrug-resistant; ND, not determined; CP, ciprofloxacin; CF, cefpime; CT, ceftriaxone; AK, amikacin; TDN, titanium dioxide nanoparticles; CP\TDN, combination of ciprofloxacin and titanium dioxide nanoparticles; CT\TDN, combination of ceftriaxone and titanium dioxide nanoparticles; CF\TDN, combination of cefpime and titanium dioxide nanoparticle.

The mechanism by which the tested nanoparticles and the bacterial cells interact depends on the interaction between the negative charges of the bacterial cells and the positive charge of metal oxides. As a result, electromagnetic skirmishes were created between bacterial cells and metal oxide surfaces. Furthermore, nanoparticles release ions that can react with the –SH group of proteins that control the transporting of material, reducing their permeability.[7,9,44]

Many studies detected antifungal and antibacterial activities of TDN as Duymaz et al[45] and Alhadrami et al.[46] Thomas (2016) showed that antimicrobial activity of toothpastes and mouthwashes against dental plaque-causing organisms were enhanced by adding TDN.[47] Recently many studies reported that the wound dressing with TDN enhanced wound healing due to its antimicrobial and cell growth stimulation features.[48–52]

In contrast to our findings, Kotlhao et al (2017) reported that TDN had no antibacterial activity against *Pseudomonas aeruginosa*[53] which may be due to the reduction in porosity of Gram-negative cell wall or existence of efflux pump that causes a decrease in intracellular concentrations of these compounds, resulting in a drastic reduction of biocidal activity.[54]

## The Effect of TDN and Antibiotics on MDR *P. aeruginosa* Isolates

The antimicrobial resistance of the 25 MDR P. aeruginosa against various antibiotics was found to be reduced in the presence of TDN. The checkerboard titration method revealed that there was a marked decrease in MICs of commercial antibiotics in combination with TDN nanoparticles. This case was called the synergistic effect. The synergistic effect between TDN and the antibiotics was evaluated by calculating the FIC index. The effect of TDN in combination was found to be synergistic.

Present results showed that using antibiotics in combination with nanoparticles increases the therapeutic activity against the resistant strains. The presence of antibiotics with TDN increases the concentration of antibiotics at the site of infection and the binding of bacteria to antibiotics.[55]

Many studies have shown that combining TDN potentiate the antimicrobial action of different classes of antibiotics.[2,7-9] FICI is an indicator of the degree of interaction between TDN with commercial antibiotics for the 25 MDR *P. aeruginosa* strains. The FICI of the combination of TDN plus cefepime shows synergistic effect to all isolates (100%), ceftriaxone; showing synergistic effect against 24 strains (96%) and additive effect against one strain (4%); ciprofloxacin showing synergistic effect against 20 strains (80%) and additive effect against three (12%) and two strains were not evaluated using the combination of TDN and ciprofloxacin as both were sensitive to ciprofloxacin; amikacin, showing synergistic effect against 22 strains (88%) and additive effect against three (12%) isolates as shown in Table 3. Masoumi et al (2018) reported the additive effect of nano-TiO$_2$ and nano-ZnO combinations (FIC = 0.95) against both *Acentobacte baumannii* and *K. pneumoniae* strains; whereas, this combination showed an indifferent effect against *P. aeruginosa* isolates (FIC > 2).[54]

## Conclusion

The addition of TDN to the tested antibiotics enhances the therapeutic activity of these antibiotics. So, the preparation of topical pharmaceutical dosage forms containing a combination of antibiotics and TDN will help in the eradication of MDR *P. aeruginosa*.

## Disclosure

The authors report no conflicts of interest in this work.

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