# Evaluation of antibiotics as potential antiproliferative agent against triple-negative breast cancer: a cell-based repurposing study

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**Background/aim**
Drug repurposing is a well-known strategy that involves identifying new therapeutic applications for existing medications. Given the global burden of breast cancer, this study aims to investigate the potential of repurposing antibiotics as a potential treatment option for this disease.
**Patients and methods**
Cytotoxic activity of about 50 antibiotics against triple-negative breast cancer cell line (MDA-MB231) was investigated using a 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The potency of these antibiotics was compared with doxorubicin as positive control while their specificity was assessed using the noncancerous cell line (BJ-1). A three-dimensional model was also used for determining the penetration power of the strongest antibiotics and an acid phosphatase assay was used to evaluate their cytotoxic effects. Moreover, gene expression analysis of P53, Bcl-2, and Bax was performed using real-time PCR.
**Results**
A screening assay was conducted to evaluate the cytotoxic effects of 50 antibiotics against MDA-MB-231 breast cancer cells. At a concentration of 100 μM, 19 antibiotics demonstrated significant cytotoxicity reducing cell viability by more than 50% after 48 h and nonsignificant affecting on normal cells. Additionally, two of these antibiotics coded with S44 and S49, exhibited potent anticancer activity with high penetration power, as evidenced by their ability to shrink three-dimensional spheroids of MDA-MB-231 cells with diameters less than untreated cells. The real-time PCR showed a significant upregulation ($P<0.5$) in the *P53* and Bax levels, while Bcl-2 expression was down regulated significantly ($P<0.5$) by S44 and S49.
**Conclusion**
The findings suggest that repurposing antibiotics may offer promising therapeutic avenues for triple-negative breast cancer treatment. Further investigations are warranted to elucidate the underlying mechanisms of action and evaluate the clinical potential of these compounds.

**Keywords:**
antibiotics, breast cancer, human cancer cell lines, repurposing of drugs

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## Introduction

Drug repurposing, which involves identifying new uses for existing medications, offers a promising viable strategy in cancer drug discovery and antibiotic development. It potentially accelerates drug development and reduces costs compared with traditional methods of creating new drugs [1]. It has already been found to be safe in preclinical experiments and in humans [2]. Since most preclinical testing, safety evaluations, and, in certain situations, formulation development, will already have been finished, the time required for drug development can be shortened [3]. These advantages of reduced risk, accelerated development, and potential cost savings can lead to a more efficient return on investment in drug repurposing [4].

The normal breast contains, besides fat cells, a network of lobes that are made up of tiny, tube-like structures called lobules that contain milk glands. Tiny ducts connect the glands, lobules, and lobes, and carry milk from the lobes to the nipple. Most breast cancers begin in the cells lining the milk ducts and are called ductal carcinomas [5–7].

Lobular carcinoma is the second most commonly seen form that begins in the lobules. The cancer's location, growth rate, and the probability that it has spread are all

indicated by the stage. There are generally 5 stages for breast cancer: stage 0 (zero), which is also known as noninvasive cancer or ductal carcinoma in situ (DCIS), and stages I through IV (1 through 4). Every person's breast cancer is distinctive, and the biology and behaviors associated with the disease determine the treatment plan [8].

The initial course of treatment for cancer in its earlier stages is typically surgery to remove the tumor and any surrounding lymph nodes. After surgery, additional treatment is typically administered to reduce the chance of the cancer returning. This treatment may include chemotherapy, radiation therapy, hormone therapy, or targeted therapy. To reduce the growth of the tumor, these treatments may also be administered before surgery [9].

Several noncancer drugs, including antibiotics, have shown potential anticancer properties by targeting common molecular pathways linking diseases. The most successful example of drug repurposing are repurposing of Raloxifene as an anticancer (Breast cancer) while its original indication was Osteoporosis. Similarly, aspirin, which has long been used to treat pain, has shown promise as an anticancer agent, particularly for colorectal cancer. Other notable examples of drug repurposing include sildenafil (Viagra), originally developed for erectile dysfunction, which has also been found effective for pulmonary hypertension, and thalidomide, which was initially used for leprosy but is now approved for multiple myeloma [10]. Therefore, the aim of the present work is the repurposing of different widely used antibiotics for their anticancer effect against triple-negative of breast cancer cell line MDA-123).

## Patient and methods

### Sampling and study design

Fifty antibiotics were randomly purchased from commercial drug stores in Cairo, Egypt, as listed in Table 1.

### Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study has been approved by the Ethical Committee of the National Research Centre with approval number 19/289.

## Methods

### Cell lines

The estrogen-negative MDA-MB-231 human cancer cell line and Skin Fibroblast (BJ-1) normal cells were

**Table 1 Antibiotics drugs used under investigation**

| Type | Drug name | Code | Type | Drug name | Code |
|---|---|---|---|---|---|
| Coated tablet | Cephalexin | S26 | Ampoule | Ampicillin | S1 |
| Tablet | Amoxicillin and flucloxacillin | S27 | Ampoule | Cephradine | S2 |
| Coated tablet | Levofloxacin | S28 | Ampoule | Ceftriaxone | S3 |
| Coated tablet | Cephalexin | S29 | Ampoule | Ceftazidime | S4 |
| Ampoule | Ceftriaxone | S30 | Ampoule | Benzathinbenzy lpenicilline | S5 |
| Ampoule | Cephradine | S31 | Ampoule | Cefoperazone | S6 |
| Ampoule | Sulbactam and Ampicillin | S32 | Ampoule | Cefotaxime sodium | S7 |
| Ampoule | Amoxycillin and Flucloxacillin | S33 | Ampoule | Sulbactam/Ampicilln | S8 |
| Capsules | Chloramphenicol | S34 | Ampoule | Clindamycin | S9 |
| Coated tablet | Gemifloxacin | S35 | Ampoule | Ciprofloxacin | S10 |
| Coated tablet | Clarithromycin | S36 | Ampoule | Gentamicin | S11 |
| Coated tablet | Cefadroxil monohydrate | S37 | Capsules | Cefdinir | S12 |
| Ampoule | Benzathinebenzylpenicillin | S38 | Coated tablet | Zithromycin | S13 |
| Ampoule | Ceftazidine | S39 | Tablet | Ofloxacin | S14 |
| Ampoule | Clindamycin | S40 | Coated tablet | Spiramycin | S15 |
| Coated tablet | Linezolid | S41 | Tablet | Neomycin sulphat | S16 |
| Coated tablet | Moxifloxacin | S42 | Coated tablet | Amoxicillin and Clavulanic acid | S17 |
| Coated tablet | Rifaximin | S43 | Coated tablet | Moxifloxacin | S18 |
| Capsules | Doxycycline | S44 | Capsules | Chloramphenicol | S19 |
| Ampoule | Cefoperazone | S45 | Coated tablet | Clarithromycin | S20 |
| Tablet | Neomycin sulphate | S46 | Coated tablet | Rifaximin | S21 |
| Ampoule | Cefotaxime Sodium | S47 | Capsules | Doxycycline | S22 |
| Bottle | Levofloxacin | S48 | Coated tablet | Linezolid | S23 |
| Coated tablet | Nitazoxanide | S49 | Coated tablet | Cefadroxil monohydrate | S24 |
| Coated tablet | Tedizolid phosphate | S50 | Coated tablet | Gemifloxacin | S25 |

obtained from the American Type Culture Collection (USA). Cells were cultured in DMEM-12 medium supplemented with fetal bovine serum, penicillin, streptomycin, L-glutamine, and nonessential amino acids. The cultures were maintained at 37°C in a humidified incubator with 5% $CO_2$ [11].

#### Cytotoxicity of antibiotics on MDA-MB231 and BJ-1 monolayers

The anticancer activity of the selected drugs was evaluated against MDA-MB231 human breast carcinoma cells and BJ-1 normal foreskin fibroblast cells.

#### MTT assay [3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]

MDA-MB231 and BJ-1 cells were seeded into 96-well plates at a density of 10×103 and $50\times10^3$ cells per well, respectively. After 24 h, the cells were treated with a final concentration of 100 ppm of the tested drugs in triplicate. The cells were incubated for 48 h, with 1 μM doxorubicin as a positive control and 0.5% DMSO as a negative control. Cytotoxicity was assessed using the 3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described and was calculated according to the following formula: [1-(av(x))/(av (NC))]*100. Where: Av: average, X: absorbance of sample, NC: absorbance of negative control. Absorbance was measured at 595 nm with reference 690 nm [10].

#### Determination of $IC_{50}$ values

Drugs demonstrating at least 50% cytotoxicity against breast cancer cells were selected for further analysis. Dose-response studies were conducted at various concentrations and the resulting data was used to calculate IC50 values using probit analysis with SPSS software.

#### Cytotoxicity bioassay on the estrogen negative MDA-MB-231 multi-cellular spheroids

Three-dimensional (3D) multicellular tumor spheroids (MCTs) are becoming a valuable tool in cancer research due to their ability to better replicate the *in vivo* tumor microenvironment compared with traditional 2D cell cultures. MCTs exhibit growth dynamics and cell-cell interactions that closely resemble those of *in-vivo* tumors, providing a promising model for studying tumor biology and evaluating therapeutic efficacy. Drugs that demonstrated significant cytotoxicity (≥60%) against MDA-MB-231 breast cancer cells in 2D culture were further evaluated in 3D MCTs. Selected drugs were added to triplicate wells of 3D spheroids at a final concentration of 100 μg/ml and incubated for 7 days. Cisplatin and DMSO served as positive and negative controls, respectively. Cytotoxicity was measured using the acid phosphatase assay. Treated spheroids were washed, lysed, and incubated with p-nitrophenyl phosphate. The reaction was terminated, and absorbance was measured at 405 nm. Cytotoxicity was calculated using the formula: % cytotoxicity = [1–(average sample absorbance)/(average negative control absorbance)] x 100. Drugs exhibiting significant activity on 3D spheroids at 100 μg/ml underwent dose-response studies at various concentrations (100, 50, 25, and 12.5 μg/ml) [12].

#### Phase contrast microscopy and image analysis

Images of all spheroids were captured on day 5 (start of treatment) and day 7 (after treatment) using an Olympus inverted microscope equipped with an Olympus SC100 camera (Germany) and cellSens software. The images were used to evaluate spheroid growth.

#### Quantitative real time PCR for P53, Bcl-2, and Bax

MDA-MB-231 cells were treated with their respective $IC_{50}$ concentrations of promising antibiotics for 48 h. Gene expression analysis of P53, Bcl-2, and Bax was performed using real-time PCR.

#### RNA isolation

The assay was carried out according to the methodology described by Oliveira *et al.* [12] In brief, MDA-MB-231 cells were seeded into a six-well plate at a density of 3×10^5 cells per well and incubated for 24 h. The IC50 dose of promising antibiotics was then added to each well, except for the control well. Total RNA was isolated using the Trizol reagent protocol. The cells were collected and centrifuged, followed by RNA isolation using the RNeasy extraction kit and cDNA synthesis using the TranScriba First Strand cDNA Synthesis kit.

#### Reverse transcription (RT) reaction

RT-PCR analysis was carried out using specific primers for the target genes. Gene expression levels were normalized to beta-actin as a housekeeping gene. The RT reaction involved an initial incubation at 50°C for 45 min to synthesize cDNA, followed by real-time PCR amplification cycles (95°C for 10 s and 60°C for 60 s) on a Rotor-Gene 3000 system. A negative control was included in each run to assess primer specificity and contamination, using primer sequences as in Table 2 [13].

**Table 2 Primers for the target genes P53, Bcl-2, and Bax**

| | |
|---|---|
| p53 F | 5'-CCCCTCCTGGCCCCTGTCATCTTC-3' |
| p53 R | 5'-GCAGCGCCTCACAACCTCCGTCAT-3' |
| Bcl-2 F | 5'-CCTGTGGATGACTGAGTACC-3' |
| Bcl-2 R | 5'-GAGACA GCC AGG AGA AAT CA-3' |
| Bax F | 5'-GTTTCA TCC AGG ATC GAG CAG-3' |
| Bax R | 5'-CATCTT CTT CCA GAT GGT GA-3' |
| β-actin F | 5'-GTGACATCCACACCCAGAGG-3' |
| β-actin R | 5'-ACAGGATGTCAAAACTGCCC-3' |

### Statistical analysis

Statistical analysis and graph plotting for in-vitro cell line studies were performed using Microsoft Excel and GraphPad Prism 8 software (GraphPad Software, San Diego, California, USA). All data were collected in triplicate and expressed as the mean ± standard error (SE) from three independent experiments. The $IC_{50}$ was calculated by linear regression analysis using the SPSS program. ANOVA test was used to compare between more than two groups with parametric data. Statistical significant was considered at *P* value less than 0.05.

## Results

### Cytotoxicity of antibiotics on MDA-MB231 monolayers

Nineteen of the tested antibiotics exhibited cytotoxicity rates exceeding 50% against MDA-MB231 breast cancer cells at a concentration of 100 μm after 48 h. The remaining antibiotics demonstrated low cytotoxic activity against this cell line, as shown in Table 3. These promising antibiotics were introduced to further investigation at different concentrations to calculate their $IC_{50}$.

### Determination of $IC_{50}$values

The results in Table 4 showed that seven antibiotics only from the nineteen promising antibiotics demonstrated less activity against normal cells and have a significant selectivity index. These antibiotics were further tested in 3D culture systems.

### Cytotoxicity of promising antibiotics on 3D (spheroid model) for MDA-MB231 and BJ-1

The results represented in Table 5 summarize the effects of seven promising antibiotics on 3D model of both MDA-MB231 and BJ-1. Only two of the seven antibiotics tested (S44 and S49) demonstrated significant effectiveness against cancer cells while remaining safe for normal cells. Moreover, the graph of the 3D model of the MDA-MB231 cell line showed that only S44 and S49 have diameter around 170 to 190 μm otherwise the control ranged from 270 to 290 μm in diameter. Moreover, only S44 and S49 showed cytotoxic effects more than Cisplatin which is the positive control (Fig. 1).

### Comet assay

The DNA damage was assessed by performing a comet assay on MDA-MB-231 cell line before and after applying antibiotics coded with S44, S49 and doxorubicin (positive control). The results showed

**Table 3 Percentage inhibition of Antibiotic drugs against Human breast cancer cell line (MDA-MB231) at 100 μm after 48 h**

| Sample code | [+] Cytotoxicity at 100 μm against MDA-MB231 after 48h | Sample code | [+] Cytotoxicity at 100 μm against MDA-MB231after 48 h | Sample code | [+] Cytotoxicity at 100 μm against MDA-MB231 after 48 h |
|---|---|---|---|---|---|
| S1 | 25.5±3.9 | S18* | 69.9±5.14 | S35* | 87.9±7.86 |
| S2 | 18.3±2.1 | S19 | 23.2±1.82 | S36* | 59.2±5.23 |
| S3 | 8.2±0.79 | S20* | 78.6±6.96 | S37 | 12.2±0.94 |
| S4 | 28.1±2.3 | S21 | 20.5±1.69 | S38* | 73.9±6.43 |
| S5* | 90.3±8.7 | S22* | 87.4±8.16 | S39 | 5.8±0.63 |
| S6 | 0±0.0 | S23 | 36.7±3.79 | S40 | 46.5±5.62 |
| S7* | 92.1±0.82 | S24 | 12.3±0.98 | S41* | 57.1±4.79 |
| S8 | 39.3±4.2 | S25* | 59.7±5.88 | S42 | 35.5±3.11 |
| S9 | 15±0.9 | S26 | 26.8±2.79 | S43 | 41.3±3.87 |
| S10 | 0±0.0 | S27* | 56.8±5.43 | S44* | 92.7±6.98 |
| S11* | 61.2±5.1 | S28* | 54.2±4.89 | S45 | 15.8±1.21 |
| S12 | 29.2±2.5 | S29* | 72.6±6.99 | S46 | 10.8±0.87 |
| S13 | 20.2±1.87 | S30 | 8.11±0.83 | S47 | 6.76±5.14 |
| S14 | 25.9±2.18 | S31 | 5.8±0.47 | S48 | 13.5±0.98 |
| S15* | 78.9±4.21 | S32 | 9.72±0.86 | S49* | 69.6±3.99 |
| S16 | 35.8±2.99 | S33 | 6.18±0.57 | S50* | 54.9±2.76 |
| S17 | 21.8±1.89 | S34* | 59.9±4.89 | | |

[+]: Data are represented as mean±SE (Each sample was done in triplicate). *: Antibiotics within the same column exhibited more potent cytotoxic effects, with inhibition rates exceeding 50%.

**Table 4 $IC_{50}$ of nineteen antibiotics against MDA-MB231 and percentage inhibition of the antibiotics on BJ1 after 48h**

| Sample code | [+] $IC_{50}$ of antibiotics against MDA-MB231 after 48h | [+] Cytotoxicity of antibiotics against BJ1 after 48h | Selectivity index |
|---|---|---|---|
| S5* | 37.5±2.6 | 3.2±0.1 | 2.7 |
| S7* | 29.9±1.9 | 2.3±0.2 | 3.3 |
| S11 | 86.1±5.6 | 6.5±0.6 | 1.16 |
| S15 | 70.7±6.7 | 7.1±0.4 | 1.4 |
| S18 | 77.7±4.5 | 19.2±1.2 | 1.3 |
| S20 | 71.0±8.1 | 31.9±2.6 | 1.4 |
| S22* | 13.7±0.9 | 12.9±1.5 | 7.3 |
| S25 | 87.0±8.1 | 25.1±2.6 | 1.14 |
| S27 | 90.2±6.4 | 15.9±2.4 | 1.2 |
| S28 | 92.1±5.7 | 11.8±1.8 | 1.1 |
| S29 * | 8.1±0.7 | 2.4±0.3 | 12.3 |
| S34 | 86.9±7.2 | 13.5±1.2 | 1.2 |
| S35* | 14.3±1.1 | 12.8±2.1 | 7 |
| S36 | 88.1±3.7 | 30.8±2.8 | 1.13 |
| S38 | 61.1±4.2 | 14.7±0.9 | 1.63 |
| S41 | 89.4±6.2 | 34.2±2.4 | 1.1 |
| S44* | 14.4±0.4 | 18.8±0.9 | 6.9 |
| S49 * | 11.3±5.3 | 2.1±0.5 | 8.8 |
| S50 | 90.1±8.2 | 3.7±0.3 | 1.1 |

[+]: Data are represented as mean±SE. The $IC_{50}$ were calculated by linear regression analysis (SPSS program). *: Data within the same column represent the antibiotics with a greater ability to selectively target cancer cells.

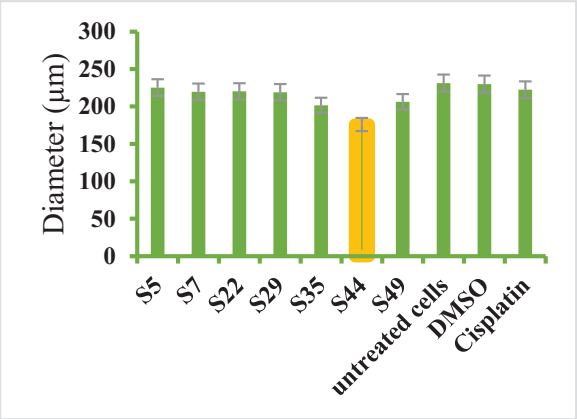
that the negative control, which was MDA-MB-231 cell before applying any drug, had a significant decrease ($P<0.05$) regarding DNA damage. Its value was 10.25 ±1.31. Nevertheless, MDA-MB-231cells after treating with S44 revealed a significant increase ($P<0.05$) with value of 21.50±1.04 and also it exceeded the value of MDA-MB-231 cells after treating with doxorubicin (positive control). Regarding to S49 its effect less than the effect of the positive control but still a significant increase ($P<0.05$), as expressed in Table 6 and shown in Fig. 2.

**Table 5 Effect of most promising antibiotic on three-dimensional (spheroid model) of both MDA-MB231 and BJ-1**

| Sample code | [+] MDA-MB231% mortality | BJ1% mortality |
|---|---|---|
| S5 | 15.9±1.5[a] | 12.1±0.9[a] |
| S7 | 23.7±1.8[a] | 13.7±1.1[a] |
| S22 | 37.2±2.1[a] | 11.7±0.7[a] |
| S29 | 42.1±1.9[a] | 9.8±0.5[a] |
| S35 | 40.3±2.1[a] | 10.3±0.8[a] |
| S44 | 73.1±3.7[b] | 2.4±0.2[b] |
| S49 | 53.9±3.4[b] | 1.9±0.1[b] |

[+]: Data are represented as mean±SE. Data with superscript letters (a, b) within the same column are significantly different at *P* greater than 0.05, using ANOVA test. The 'sample code' Colum represents the antibiotic tested, while the '% mortality' rows indicate the observed effects. A higher percentage of mortality indicates a greater effect on the cells.

**Figure 1**



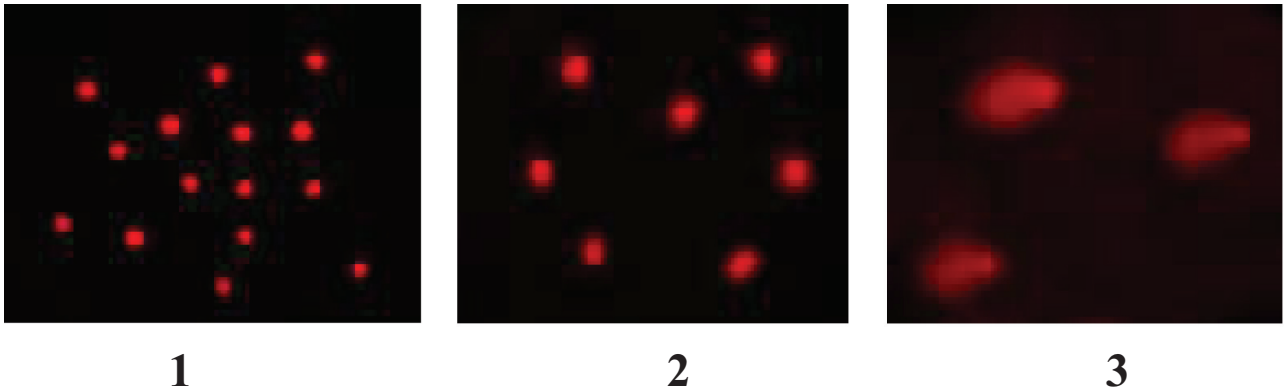

The diameter in µm of MDA-MB-231 cells spheroids after exposure to the most active antibiotic versus cisplatin.

### Evaluation of apoptosis-related genes

*Quantitative real time polymerase chain reaction for P53, Bcl-2 and Bax*

The real-time PCR showed a significant upregulation ($P<0.5$) in the *P53* level in MDA-MB-231 cell line

**Figure 2**




Visual score of normal DNA: 1(class 0), 2 (class 1 and 2) and 3 (class 3) using comet assays.

**Table 6 Visual score of DNA damage in breast cancer cell lines treated with S44 and S49**

| | Number of cells | | | Class** | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | No of samples | Analyzed * | Comets | 0 | 1 | 2 | 3 | Percentage of DNA damaged cells (Mean±SE) |
| S44 | 4 | 400 | 86 | 314 | 30 | 19 | 37 | **21.50±1.04[a]** |
| S49 | 4 | 400 | 69 | 331 | 24 | 15 | 30 | **17.25±0.9 [b]** |
| Negative (–ve) | 4 | 400 | 41 | 359 | 26 | 5 | 10 | **10.25±1.31[c]** |
| Postive (+ve) | 4 | 400 | 82 | 318 | 24 | 28 | 30 | **20.51±0.65 [a]** |

*: Number of cells examined per a group, **: Class 0= no tail; 1= tail length and lt; diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length and gt; 2X the diameter of nucleus. Data with superscript letters (a, b, c) within the same Colum are significantly different at *P* greater than 0.05, using ANOVA test.

**Table 7 Effect of S44 and S49 antibiotics in genes expression of P53, Bcl-2, and Bax**

| | Fold Change | | |
|---|---|---|---|
| Code | P53 | Bcl-2 | Bax |
| S44 | 5.3±0.41 [b] | 0.4±0.05 [c] | 5.2±3.5 [b] |
| S49 | 4.4±0.26 [b] | 1.54±0.1 [d] | 4.7±2.7 [b] |
| Control –ve | 1 [a] | 1 [a] | 1 [a] |
| *Cisplatin* | 9.0±2.8 [c] | 0.81±0.59 [b] | 10.9±4.9 [c] |

S44: Doxycycline, S49: Nitazoxanide, A value greater than 1 indicates an up regulation, while a value less than 1 indicates down regulation. Data with superscript letters (a, b, c, d) within the same Colum are significantly different at *P* less than 0.05, using ANOVA test.

treated with antibiotic coded with S44 and S49 in comparison with untreated cells with FLD 5.3 and 4.4, respectively. Regarding to Bcl-2 expression, it was found that a significant down regulation ($P<0.5$) by S44 and S49 while both of them are significantly upregulated Bax expression in MDA-MB-231 cells compared with control group (Table 7).

## Discussion

Antibiotics play a pivotal role in treating infectious diseases, significantly reducing mortality rates and enhancing overall quality of life [14]. Beyond their traditional use in treating infections, antibiotics have emerged as potential cancer therapies; however, more research is necessary to fully understand their mechanisms of action and evaluate their clinical efficacy [15–17]. To investigate the potential anticancer effects of these antibiotics, we evaluated 50 compounds against the human adenocarcinoma epithelial breast cell from Mammary gland (MDA-MB-231) breast cancer cell line using different assays.

Among the tested compounds, S44 (Doxycycline) and S49 [Nitazoxanide (NTZ)] demonstrated significant anticancer activity compared with the standard chemotherapeutic agent, cisplatin. The treatment of 3D spheroids with S44 and S49 resulted in a significant reduction in size, suggesting their potential to inhibit cancer cell growth and proliferation [18]. Our cytotoxicity assessment, utilizing both 2D and 3D cancer models, aligns with the increasing emphasis on 3D models for drug screening [9,10,19]. While 3D models are often favored for their ability to better represent the tumor microenvironment, our findings demonstrate that 2D models can still be valuable for identifying promising anticancer agents [20].

Doxycycline and NTZ, two U.S. Food and Drug Administration (FDA)-approved drugs, have demonstrated promising anticancer activity in breast cancer treatment due to their ability to target cancer cells in various stages and through different mechanisms. Doxycycline has been shown reduce tumor burden in bone metastasis models [21], inhibit cancer stem cell properties and decrease their frequency and ameliorate paclitaxel-induced stem cell enrichment in triple-negative breast cancer [22], and decrease reactive oxygen species levels [22,23]. These mechanisms contribute to its anticancer effects, making it a potential candidate for breast cancer therapy. While a small study found limited benefits for lymphedema, patients reported improved quality of life [24]. Given its low toxicity and potential to target both cancer stem cells and bulk tumor cells, doxycycline may be a valuable addition to breast cancer treatment regimens[23].

NTZ, has demonstrated potent inhibition of c-Myc activation, a key factor in tumorigenesis [25]. Additionally, it targets dormant cancer cells in hypoxic tumor regions by inhibiting mitochondrial respiration [26]. Moreover, S7 (Cefotaxime sodium), S35 (Gemifloxacin), and S44 (Doxycycline) with $IC_{50}$ values of 20.029, 13.571, and 6.961 µm, respectively, demonstrated exceptional anticancer activity among the tested compounds. These antibiotics demonstrated enhanced safety against normal BJ1 cells while maintaining potent antiproliferative activity against cancer cell lines. This align with recent studies that have explored the potential of combining these antibiotics with existing cancer treatments to offer improved therapeutic outcomes

and enhance the efficacy of biological cancer therapies [27–29].

The present findings demonstrate that promising antibiotics have an antiproliferative impact on MDA-MB-231 cells, which could be the result of the induction of apoptosis, cell cycle arrest, and/or the inhibition of growth. Since the antiproliferative effect of many naturally occurring cancer chemo-preventive agents is tightly linked to their ability to induce apoptosis [11], Therefore, this study investigated whether these promising extracts could induce apoptosis in triple-negative breast cancer cells.

At the apoptotic gene expression level, our findings showed that S44 and S49 have the potential to induce apoptosis in breast cancer cells through a combination of p53 up regulation and Bcl-2 down regulation. These findings align with previous research by Son and colleagues which demonstrated that doxycycline, a tetracycline antibiotic, exhibits cytotoxic effects against pancreatic cancer cells through various mechanisms, including the activation of pro-apoptotic genes and the suppression of anti-apoptotic genes [30]. Additionally, recent studies have explored the potential anticancer properties of NTZ and doxycycline. NTZ, has been shown to target the 20S proteasome, inhibiting multiple catalytic subunits and promoting cell cycle arrest and apoptosis in cancer cells [31]. It also interferes with crucial metabolic and pro-death signaling pathways, including drug detoxification, autophagy, and c-Myc inhibition [32].

## Conclusion

In conclusion, several antibiotics exhibited significant anticancer activity against breast cancer cells while maintaining a favorable safety profile. These compounds targeted various cancer cell pathways, including apoptosis. This research contributes to the growing body of evidence supporting the potential of repurposing antibiotics for cancer therapy. Future investigations may lead to the development of novel and effective therapeutic strategies.

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Author contributions: K.M.: Performing the molecular biology techniques, cell culture methodology and manuscript preparation, S.M.E.-H.: Study design, performing cell culture methodology, and final manuscript proofing. A.A.F.S.: Performing the molecular and cell culture methodology. Z.A.E.: statistical analysis and writing the initial draft of the manuscript. E.M.Y.: Conducting molecular and cell culture experiments, and contributing to manuscript preparation. All authors revised and approved the final manuscript.

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#### Conflicts of interest

There are no conflicts of interest.

## References

1 Kirtonia A, Gala K, Fernandes SG, Pandya G, Pandey AK, Sethi G, *et al.* Repurposing of drugs: An attractive pharmacological strategy for cancer therapeutics. Seminars in Cancer Biology 2021; 68:258–278.

2 Spellicy SE, Hess DC. Recycled Translation: Repurposing Drugs for Stroke. Transl Stroke Res 2022; 13:866–880.

3 Moreira-Silva F, Camilo V, Gaspar V, Mano JF, Henrique R, Jerónimo C. Repurposing Old Drugs into New Epigenetic Inhibitors: Promising Candidates for Cancer Treatment. Pharmaceutics 2020; 12:410.

4 Talevi A, Bellera CL. Challenges and opportunities with drug repurposing: finding strategies to find alternative uses of therapeutics. Expert opinion on drug discovery 2020; 15:397–401.

5 El-Hallouty SM, Fayad W, Meky NH, EL-Menshawi BS, Wassel GM, Hasabo AA. In vitro anticancer activity of some Egyptian plant extracts against different human cancer cell lines. Int J Pharmtech Res 2015; 8:267–272.

6 Shah R, Rosso K, Nathanson SD. Pathogenesis, prevention, diagnosis and treatment of breast cancer. World J Clin Oncol 2014; 5:283–298.

7 Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. Nature Reviews Drug Discovery 2004; 3:673–683.

8 Fayad W, El-Hallouty SM, El-Manawaty MA, Mounier MM, Soliman AFM, Yousry AA, *et al.* A systematic multicellular spheroids screening approach lead to the identification of antineoplastic activity in three different plant extracts from the Egyptian flora. J App Pharm Sci 2017; 7:013–022.

9 Kuo CT, Wang JY, Lin YF, Wo AM, Chen BP, Lee H. Three-dimensional spheroid culture targeting versatile tissue bioassays using a PDMS-based hanging drop array. Scientific Reports 2017; 7:4363.

10 Osman ME, Eid MM, Khattab OH, El-Hallouty SM, El-Manawaty M, Mahmoud DA. In vitro cytotoxicity of biosynthesized Ag/CS NP against MCf7, PC3 and A549 cancer cell lines. Int. J. Pharm. Tech. Res 2015; 8:1011–1017.

11 Mahmoud K, AF SA, Walid F, Youssef EM. Cell based and In vivo systematic evaluation of some Egyptian plant extracts targeting breast cancer. Toxicon 2024; 244:107752.

12 Oliveira SFVD, Ganzinelli M, Chilà R, Serino L, Maciel ME, Urban CDA, *et al.* Characterization of MTAP gene expression in breast cancer patients and cell lines. XX. 2016; 11:e0145647.

13 Yu YJ, Majumdar AP, Nechvatal JM, Ram JL, Basson MD, Heilbrun LK, Kato I. Exfoliated cells in stool: A source for Reverse Transcription-PCR-based analysis of biomarkers of gastrointestinal cancer. *Cancer* Epidemiol Biomarkers Prev 2008; 17:455–458.

14 Artem'Eva OA, Pereselkova DA, Fomichev YP. Dihydroquercetin, the bioactive substance, to be used against pathogenic microorganisms as an alternative to antibiotics. Agric Biol 2015; 50:513–519.

15 Booser DJ, Hortobagyi GN. Anthracycline antibiotics in cancer therapy. Focus on drug resistance. Drugs 1994; 47:223–258.

16 Xia D, Yang X, Liu W, Shen F, Pan J, Lin Y, *et al.* Over-expression of CHAF1A in Epithelial Ovarian Cancer can promote cell proliferation and inhibit cell apoptosis. Biochem biophys res commun 2017; 486:191–197.

17 Nelson ML, Levy SB. The history of the tetracyclines. Ann New York Acad Sci 2011; 1241:17–32.

18 Cheng G, Tse J, Jain RK, Munn LL. Micro-Environmental Mechanical Stress Controls Tumor Spheroid Size and Morphology by Suppressing Proliferation and Inducing Apoptosis in Cancer Cells. PLoS ONE 2009; 4: e4632.

**19** Pal S, Chatterjee N, Das AK, McClements DJ, Dhar P. Sophorolipids: A comprehensive review on properties and applications. Adv colloid interface sci 2023; 313:102856.

**20** Melissaridou S, Wiechec E, Magan M, Jain MV, Chung MK, Farnebo L, Roberg K. The effect of 2D and 3D cell cultures on treatment response, EMT profile and stem cell features in head and neck cancer. Cancer Cell Int 2019; 19:16.

**21** Duivenvoorden WC, Popović SV, Lhoták S, Seidlitz E, Holger WH, Richard GT, Gurmit S. Doxycycline decreases tumor burden in a bone metastasis model of human breast cancer. Cancer Res 2002; 62:1588–1591.

**22** Zhang L, Xu L, Zhang F, Vlashi E. Doxycycline inhibits the cancer stem cell phenotype and epithelial-to-mesenchymal transition in breast cancer. Cell cycle (Georgetown, Tex) 2017; 16:737–745.

**23** Lin CC, Lo MC, Moody RR, Stevers NO, Tinsley SL, Sun D. Doxycycline targets aldehyde dehydrogenase positive breast cancer stem cells. Oncol Rep 2018; 39:3041–3047.

**24** Brown S, Dayan JH, Coriddi M, McGrath L, Kataru RP, Mehrara BJ. Doxycycline for the treatment of breast cancer-related lymphedema. Front. Pharmacol 2022; 13:1028926.

**25** Fan-Minogue H, Bodapati S, Solow-Cordero D, Fan A, Paulmurugan R, Massoud TF, *et al.* A c-Myc activation sensor-based high-throughput drug screening identifies an antineoplastic effect of nitazoxanide. Mol Cancer Ther 2013; 12:1896–1905.

**26** Senkowski W, Zhang X, Olofsson MH, Isacson R, Höglund U, Gustafsson M, *et al.* Three-Dimensional Cell Culture-Based Screening Identifies the Anthelmintic Drug Nitazoxanide as a Candidate for Treatment of Colorectal Cancer. Mol Cancer Ther 2015; 14:1504–1516.

**27** He X, Yao Q, Fan D, Duan L, You Y, Lian W, *et al.* Combination of Cefotaxime and Cisplatin Specifically and Selectively Enhances Anticancer Efficacy in Nasopharyngeal Carcinoma. Curr Cancer Drug Targets 2023; 23:572–584.

**28** Al-Nuaimi A, Al-Hiari Y, Kasabri V, Haddadin R, Mamdooh N, Alalawi S, Khaleel S. A Novel Class of Functionalized Synthetic Fluoroquinolones with Dual Antiproliferative – Antimicrobial Capacities. Asian Pac J Cancer Prev 2021; 22:1075–1086.

**29** Halawani EM, Hassan AM, Gad El-Rab SMF. Nanoformulation of Biogenic Cefotaxime-Conjugated-Silver Nanoparticles for Enhanced Antibacterial Efficacy Against Multidrug-Resistant Bacteria and Anticancer Studies. Int J Nanomedicine 2020; 18:1889–1901.

**30** Son K, Fujioka S, Iida T, Furukawa K, Fujita T, Yamada H, *et al.* Doxycycline induces apoptosis in PANC-1 pancreatic cancer cells. Anticancer Res 2009; 29:3995–4003.

**31** Lü Z, Li X, Li K, Ripani P, Shi X, Xu F, *et al.* Nitazoxanide and related thiazolides induce cell death in cancer cells by targeting the 20S proteasome with novel binding modes. Biochem Pharmacol 2022; 197:114913.

**32** Di Santo N, Ehrisman J. A functional perspective of nitazoxanide as a potential anticancer drug. Mutation research 2014; 768:16–21.