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## Antimicrobial and Antibiofilm Activities of Paracetamol in Combination with Various Antimicrobials



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### Abstract

**Background and Aims:** In recent years, very few new antimicrobial agents have been approved and used for the treatment of infectious diseases. Paracetamol (acetaminophen) is one of the nonsteroidal anti-inflammatory drugs with analgesic and antipyretic effects, which is frequently preferred and used safely in our country and in many other countries. In addition, because it is the most frequently prescribed drug after antibiotics and is often administered with antimicrobial therapy, understanding the interactions between these two drug classes is extremely important for drug repurposing.

**Methods:** For this purpose, the effects of paracetamol alone or in combination with various antimicrobial agents and their activities on adhesion and biofilm formation were investigated against various standard bacteria and yeasts.

**Results:** The minimum inhibitory concentration of paracetamol against standard strains was found 10,000 µg/mL, and the minimum bactericidal and fungicidal concentrations were found 10,000 - > 20,000 µg/mL. Cefepime and paracetamol combinations against *Escherichia coli* and *Achromobacter xylosoxidans*, and gentamicin and paracetamol combinations against *Klebsiella pneumoniae* and *Acinetobacter baumannii* showed synergistic effect. No antagonism was observed. According to the biofilm adhesion and formation inhibition assays, it was found that paracetamol was more effective against gram-negative bacteria than gram-positive bacteria and yeasts.

**Conclusions:** In conclusion, this study proved that paracetamol, which is one of the most common analgesic and antipyretic agents in clinical use for many years, has antimicrobial and antibiofilm activity and can show synergistic effect in combination with various antibiotics.

### Keywords


Paracetamol · Antimicrobial activity · Combination · Biofilm · Checkerboard



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## INTRODUCTION

The discovery, commercialization and routine application of antimicrobial compounds to treat infections has revolutionised modern medicine and changed the therapeutic paradigm (Munita & Arias, 2016). Although there are many antimicrobial agents to choose from for the treatment of infectious diseases, antimicrobial resistance exists against almost all of them, and this resistance can appear shortly after a new drug is approved for use (Reygaert, 2018). With the "one health approach" limiting the unnecessary use of antimicrobials, new pathogen-targeted therapies are increasingly needed to combat the multifaceted resistance potential of microorganisms (Christaki et al., 2020).

Nowadays, antimicrobials are frequently overused because of the relatively low incidence of toxicity and the perception of maximising benefit with minimal risk. This overuse has led to multidrug-resistant, extensively drug-resistant and pandrug-resistant organisms. Unfortunately, the discovery of new agents has not kept pace with the threats posed by rapidly emerging microorganisms with antimicrobial resistance (Spellberg et al., 2011). In order to eliminate this important health threat, it is necessary to establish resistance surveillance systems, to implement resistance management practices and to control the use of antibiotics in medicine and agriculture. The most important of these practices is the development of new and effective antibiotics. However, in recent years, very few antibiotics have been approved by the FDA and have been used for the treatment of infectious diseases.

To this end, "Drug-repurposing" (or drug re-profiling) is currently being used to study the antimicrobial properties of other drugs, such as non-antimicrobial non-steroidal anti-inflammatory drugs (NSAIDs), in order to minimise the time and economic costs of new drug discovery processes (Miró-Canturri et al., 2019). Recent research aims to elucidate/explore the antimicrobial effects of NSAIDs and other similar molecules (Yadav et al., 2021).

Paracetamol, also known as acetaminophen, on the World Health Organization (WHO) "List of Essential Medicines", is a drug with a unique clinical pharmacological profile in the category of NSAIDs with strong antipyretic and analgesic effects but weak anti-inflammatory activity (Botting, 2000; Brune et al., 2015; World Health Organization, 2021). Paracetamol, one of the most widely used NSAIDs, is recommended as first-line treatment for most cases of pain and fever. It has a favorable safety profile, which will be extremely important at all ages, especially in the elderly, in pregnancy, and even in infants and newborns (Freo et al., 2021). In addition, it has antimicrobial activity and studies have shown that it can enhance

the activity of antimicrobial agents by exerting a synergistic antibacterial effect with antibiotics, reducing biofilm production and other virulence factors, or altering the susceptibility of pathogens to antimicrobial agents (Lagadinou et al., 2020).

Therefore, the widespread use of NSAIDs for anaesthetic and analgesic purposes, and in many cases in combination with antimicrobial agents, may offer the opportunity to increase the efficacy of antimicrobial treatments without increasing the safety risk to patients as part of antibacterial prophylaxis and treatment (Gil et al., 2020).

The current study aimed to evaluate the antimicrobial activity of paracetamol against a variety of standard gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Achromobacter xylosoxidans* and gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis* and *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis* fungi, and its effects in combination with antibiotics and antifungals, with which it is usually combined during infection. In addition, the effect of paracetamol on the adhesion of microorganisms and biofilm formation was investigated in comparison with antimicrobial agents. The results of the study contributed to a better understanding of the antimicrobial and antibiofilm effects of paracetamol, which has been one of the most preferred pain-relieving drugs for many years both in our country and all over the world.

## MATERIALS AND METHODS

### Strains and Culture Conditions

*S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 4352, *P. mirabilis* ATCC 14153, *P. aeruginosa* ATCC 27853, *A. baumannii* ATCC 19606, *A. xylosoxidans* ATCC 27061 bacterial and *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750 fungal strains were subcultured from freezer stocks onto tryptic soy agar (TSA, Difco Sparks, MD, USA) and incubated at 37 °C overnight.

### Antimicrobial Agents

Antimicrobial agents were kindly provided by their manufacturers, such as paracetamol from Atabay Kimya San. ve Tic. A.Ş.; gentamycin, ciprofloxacin, vancomycin, and linezolid from Koçak Farma İlaç ve Kimya Sanayi A.Ş.; daptomycin from Novartis Sağlık, Gıda, Tarım Ürünleri San. Tic. A.Ş.; ceftazidime from Glaxosmithkline İlaçları San. ve Tic. A.Ş.; cefepime from Sigma-Aldrich; azithromycin from Pfizer İlaçları A.Ş and as antifungal amphotericin B from Bristol-Myers Squibb.



Stock solutions of paracetamol and antimicrobials were prepared from dry powders with their recommended solvents. Frozen solutions of the antimicrobial agents were used within six months. Final concentrations of antimicrobials were prepared in cation-adjusted Mueller-Hinton broth (CAMHB, Difco Sparks, MD, USA) before use (Clinical and Laboratory Standards Institute, 2023).

## Media

To determine the minimum inhibitor concentration (MIC) and combination values using the microdilution checkerboard method, CAMHB was used for bacteria and RPMI medium (Sigma, St. Louis, MO, USA) for fungi; Tryptic soy agar (TSA, Difco Sparks, MD, USA) was used to determine the minimum bactericidal and fungicidal concentration (MBC and MFC), and brain-heart infusion broth (BHIB, Thermo Scientific, Oxoid, USA) was used to determine biofilm adhesion and biofilm formation values.

## Determination of MIC, MBC and MFC

MIC values of the antimicrobial agents and paracetamol were determined by the microdilution method reported by CLSI (Clinical and Laboratory Standards Institute, 2023; Clinical and Laboratory Standards Institute (CLSI), 2008). MIC values of; azithromycin, linezolid, vancomycin against *S. aureus*; azithromycin, daptomycin, ciprofloxacin against *S. epidermidis*; linezolid, ciprofloxacin, vancomycin against *E. faecalis*; gentamicin, cefepime, ciprofloxacin against *E. coli*, *K. pneumoniae* and *P. mirabilis*; gentamicin, ceftazidime, ciprofloxacin against *P. aeruginosa* and *A. baumannii*; gentamicin, cefepime, ciprofloxacin against *A. xylosoxidans*; and amphotericin B against *C. albicans*, *C. parapsilosis* and *C. tropicalis* strains, were investigated.

The MBC and MFC were defined as the lowest concentration of paracetamol resulting in at least a 99.9% killing of the initial inoculums (National Committee for Clinical Laboratory Standards & Barry, 1999). The experiments were repeated at least twice.

## Checkerboard Assay: Determination of the Fractional Inhibitory Concentration Index (FICI)

The effects of paracetamol in combination with antimicrobial agents against bacterial and fungal strains were determined by "microdilution checkerboard method" (Pillai et al., 2005). Each microtiter well, containing a mixture of paracetamol and antimicrobial agents at different final concentrations ranging from 2xMIC to 1/8xMIC, was inoculated with fresh culture. After an incubation period of 18-20 h at 37° C, the FIC index was calculated using the following formulas:  $FIC_A = (MIC_A$

in combination)/(MIC<sub>A</sub> alone),  $FIC_B = (MIC_B \text{ in combination}) / (MIC_B \text{ alone})$ , and the FIC index =  $FIC_A + FIC_B$ . The combination value was calculated from the highest dilution of the antimicrobial combination that produced no visible growth. Using this method, a FICI of  $\leq 0.5$  was defined as synergistic,  $> 0.5-4$  as additive and,  $> 4.0$  as antagonistic (Odds, 2003). The experiments were repeated at least twice.

## Inhibition of Biofilm Adhesion and Formation

In order to determine the effects of paracetamol and antimicrobial agents on adhesion and biofilm formation, microorganism suspensions ( $1 \times 10^6$  cfu/mL) and paracetamol or antimicrobial agents were prepared and added to the wells of flat bottom 96-well microplate. Positive control wells without paracetamol or antimicrobial agent and negative control without cells were also added to each microplate.

1/10xMIC values of paracetamol or antimicrobial agent were used for inhibition of adhesion and the plates were incubated for 1, 2 and 4 h for bacteria and 2, 4 and 6 h for yeasts; 1xMIC, 1/10xMIC, 1/100xMIC values of paracetamol or antimicrobial agent were used for inhibition of biofilm formation and the plates were incubated for 24 h (plates were prepared separately for each hour). At the end of incubation, the upper liquid in the wells was discarded and washed twice with sterile physiological buffered saline (PBS) to remove non-adherent cells. After incubation, the wells were washed twice with PBS and measured spectrophotometrically at optical density (OD) 450 nm, on a microplate reader (BioRad Novapath) (Bozkurt Guzel et al., 2019; Hacıoğlu et al., 2021). The results were analyzed with GraphPad Software program.

## RESULTS

### MIC, MBC and MFC Results

MICs of paracetamol and antimicrobial agents against various standard bacterial and fungal strains are shown in Table 1. The MIC of paracetamol against all standard strains was found 10,000 µg/mL. The MIC values of the antibiotics were 0.5 µg/mL for azithromycin and daptomycin; 1-16 µg/mL for gentamicin; 1 and 2 µg/mL for linezolid; 0.125 and 32 µg/mL for cefepime; 4 and 8 µg/mL for ceftazidime; 0.015-4 µg/mL for ciprofloxacin; and 2 µg/mL for vancomycin. The MIC value of amphotericin B was determined as 0.5 µg/mL. MBC and MFC values of paracetamol against standard bacterial and fungal strains were determined in the range of 10,000 - >20,000 µg/mL as summarized in Table 2.



**Table 1.** MIC values of paracetamol and antibiotics against standard bacteria and fungi ( $\mu\text{g/mL}$ )

Strains	APAP	AZM	DAP	GM	LZD	FEP	CAZ	CIP	VA	AMP-B
<i>S. aureus</i> ATCC 29213	10,000	0.5	-	-	1	-	-	-	2	-
<i>S. epidermidis</i> ATCC 12228	10,000	0.5	0.5	-	-	-	-	1	-	-
<i>E. faecalis</i> ATCC 29212	10,000	-	-	-	2	-	-	1	2	-
<i>E. coli</i> ATCC 25922	10,000	-	-	1	-	0.125	-	0.015	-	-
<i>K. pneumoniae</i> ATCC 4352	10,000	-	-	2	-	0.125	-	0.06	-	-
<i>P. mirabilis</i> ATCC 14153	10,000	-	-	2	-	0.125	-	0.06	-	-
<i>P. aeruginosa</i> ATCC 27853	10,000	-	-	2	-	-	4	1	-	-
<i>A. baumannii</i> ATCC 19606	10,000	-	-	8	-	-	8	4	-	-
<i>A. xylosoxidans</i> ATCC 27061	10,000	-	-	16	-	32	-	2	-	-
<i>C. albicans</i> ATCC 10231	10,000	-	-	-	-	-	-	-	-	0.5
<i>C. parapsilosis</i> ATCC 22019	10,000	-	-	-	-	-	-	-	-	0.5
<i>C. tropicalis</i> ATCC 750	10,000	-	-	-	-	-	-	-	-	0.5

--: Not studied; APAP: Paracetamol; AZM: Azithromycin; DAP: Daptomycin; GM: Gentamicin; LZD: Linezolid; FEP: Cefepime; CAZ: Ceftazidime; CIP: Ciprofloxacin; VA: Vancomycin, AMP-B: Amphotericin B

**Table 2.** MBC and MFC values of paracetamol against standard strains ( $\mu\text{g/mL}$ )

Strains	Paracetamol
<i>S. aureus</i> ATCC 29213	>20,000
<i>S. epidermidis</i> ATCC 12228	>20,000
<i>E. faecalis</i> ATCC 29212	>20,000
<i>E. coli</i> ATCC 25922	10,000
<i>K. pneumoniae</i> ATCC 4352	10,000
<i>P. mirabilis</i> ATCC 14153	10,000
<i>P. aeruginosa</i> ATCC 27853	10,000
<i>A. baumannii</i> ATCC 19606	10,000
<i>A. xylosoxidans</i> ATCC 27061	10,000
<i>C. albicans</i> ATCC 10231	>20,000
<i>C. parapsilosis</i> ATCC 22019	10,000
<i>C. tropicalis</i> ATCC 750	>20,000

### Checkerboard Results

According to the results, with a FIC index of  $\leq 0.5$  as borderline, cefepime and paracetamol combinations showed synergistic effect against *E. coli* and *A. xylosoxidans* strains, and gentamicin and paracetamol combinations against *K. pneumoniae* and *A. baumannii* strains. No antagonist effect was observed in any strain studied. The combination results are shown in Table 3.

**Table 3.** FIC index obtained with combinations of paracetamol with antimicrobial agents

Strains	AZM	DAP	GM	LZD	FEP	CAZ	CIP	VA	AMP-B
SA	0.75	-	-	0.625	-	-	-	0.625	-
SE	1.25	0.75	-	-	-	-	1.5	-	-
EF	-	-	-	0.75	-	-	2.25	0.530	-
EC	-	-	1	-	0.375*	-	0.625	-	-
KP	-	-	0.5*	-	0.75	-	1.25	-	-
PM	-	-	0.75	-	0.75	-	1.25	-	-
PA	-	-	1	-	-	1.25	1.25	-	-
AB	-	-	0.25*	-	-	1.25	0.625	-	-
AX	-	-	1.06	-	0.5*	-	0.75	-	-
CA	-	-	-	-	-	-	-	-	0.625
CP	-	-	-	-	-	-	-	-	1.031
CT	-	-	-	-	-	-	-	-	0.531

\*Synergist effect, -Not studied; AZM: Azithromycin; DAP: Daptomycin; GM: Gentamicin; LZD: Linezolid; FEP: Cefepime; CAZ: Ceftazidime; CIP: Ciprofloxacin; VA: Vancomycin; AMP-B: Amphotericin B; SA: *Staphylococcus aureus*; SE: *Staphylococcus epidermidis*; EF: *Enterococcus faecalis*; EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PM: *Proteus mirabilis*; PA: *Pseudomonas aeruginosa*; AB: *Acinetobacter baumannii*; AX: *Achromobacter xylosoxidans*; CA: *Candida albicans*; CP: *Candida parapsilosis*; CT: *Candida tropicalis*

### Inhibition of Biofilm Adhesion

The effects of paracetamol and antimicrobial agents on the adhesion of microorganisms at  $1/10 \times \text{MIC}$  were investigated and compared with the positive control (Figure 1). According to the results, paracetamol showed the highest inhibitory effect at 4 h for bacteria and at 6 h for fungi. Paracetamol showed the highest inhibitory effect on biofilm adhesion against *A. baumannii* with 20.5% inhibition rate and against *K. pneumoniae* and *A. xylosoxidans* with 14% inhibition rates (Figure 1 e, h, i). Against *S. aureus* and *E. faecalis*, linezolid was found to be more effective than paracetamol, while paracetamol was found to be more effective than antibiotics against *S. epidermidis* (Figure 1 a-c). The most effective antibiotics inhibiting the adhesion of gram-negative bacteria were gentamicin and ciprofloxacin, except for *E. coli* (Figure 1 d-i). Against *Candida* adhesion, amphotericin B was found to be more effective than paracetamol for all three *Candida* studied (Figure 1 j-l).

### Inhibition of Biofilm Formation

The effects of paracetamol and antimicrobial agents at different concentrations ( $1 \times \text{MIC}$ ,  $1/10 \times \text{MIC}$ ,  $1/100 \times \text{MIC}$  values) on biofilm formation of microorganisms were investigated and compared with the positive control, as shown in Figure 2. As expected, paracetamol and antimicrobial agents were found to be the most effective at MIC values. It was shown that



paracetamol was more effective on the inhibition of biofilm formation of gram-negative bacteria than gram-positive bacteria and yeasts. The results demonstrated that paracetamol was more effective than azithromycin in inhibiting biofilm formation against both *S. aureus* and *S. epidermidis* (Figure 2 a, b) and more effective than vancomycin against *E. faecalis* (Figure 2 c). Furthermore, it was more effective than gentamicin against *E. coli* and *A. xylosoxidans*, *P. aeruginosa* and *P. mirabilis* (Figure 2 d, f, g, i). Against *Candida*, amphotericin B was found to be more effective than paracetamol in the inhibition of biofilm formation (Figure 2 j-l).

## DISCUSSION

NSAIDs such as paracetamol, acetylsalicylic acid, diclofenac and ibuprofen have almost no antimicrobial activity at therapeutic range concentrations (Yadav et al., 2021). However, when co-administered with antibiotics, they can potentiate antimicrobial activity and increase the inhibitory effect of the administered drug by either inhibiting bacterial growth or altering the resistance mechanism (Chan et al., 2017). According to the results obtained from previous studies, it has been shown that these drugs can be effective at therapeutically achievable plasma concentrations in combination with antibiotics used for topical applications, even if it is understood that they cannot be used systematically due to toxicity. Therefore, NSAIDs can be easily formulated with some topical antimicrobial agents that can be used as solutions, gels and ointments in clinical practice and thus alternative antimicrobial preparations can be developed to reduce the use of antibiotics (Yadav et al., 2021).

In this study, the effects of paracetamol alone and in combination with various antimicrobial agents were investigated against bacterial and fungal strains. The MIC value of paracetamol was determined as 10,000 µg/mL against all strains studied. MICs of the antibiotics were 0.5 µg/mL for azithromycin and daptomycin, 1-16 µg/mL for gentamicin, 1 and 2 µg/mL for linezolid, 0.125 and 32 µg/mL for cefepime; 4 and 8 µg/mL for ceftazidime; 0.015-4 µg/mL for ciprofloxacin and 2 µg/mL for vancomycin. The MIC value of amphotericin B was determined as 0.5 µg/mL. The MBC and MFC values of paracetamol were determined in the range of 10,000 - > 20,000 µg/mL.

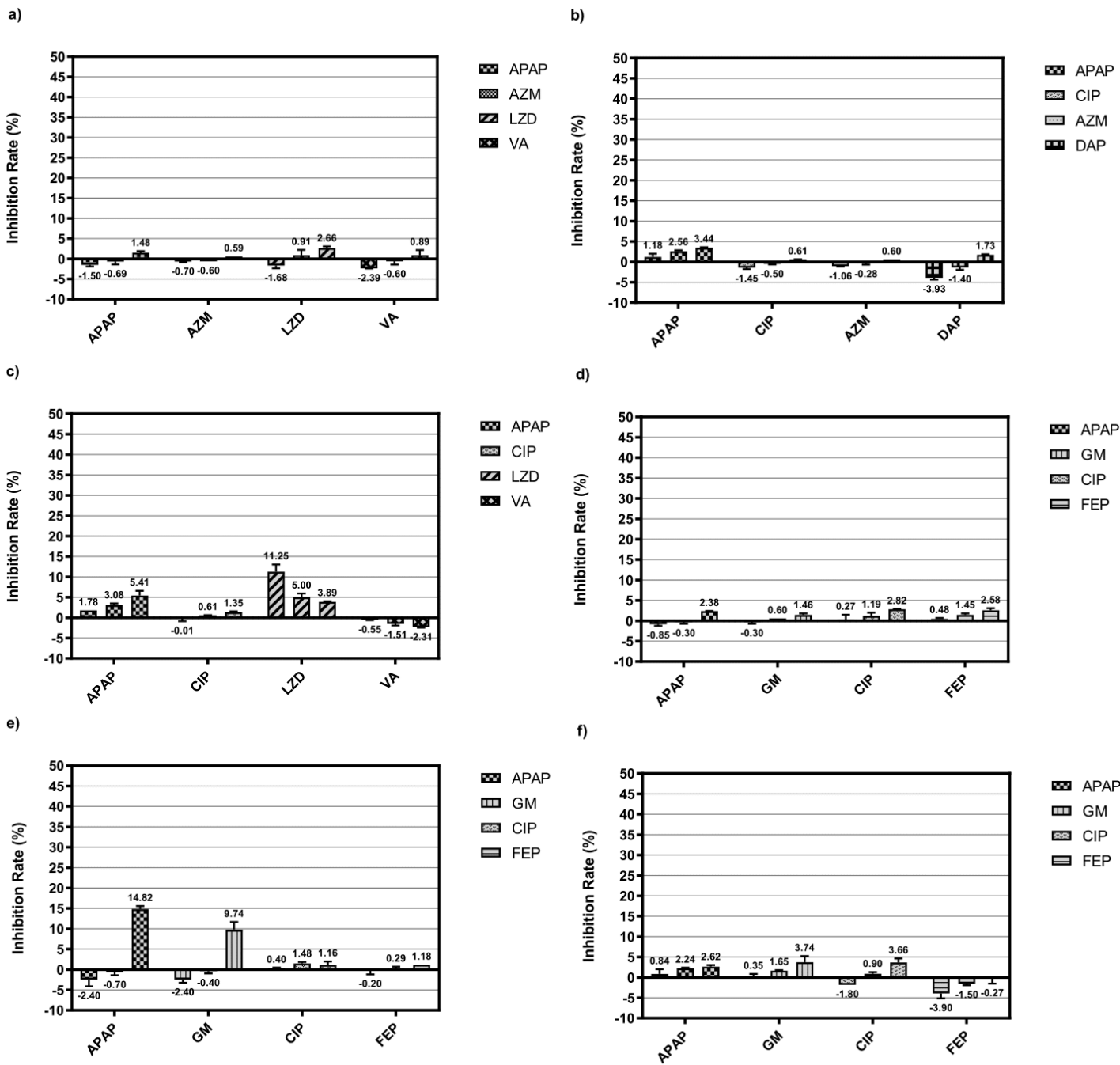
Previous researches have also shown that NSAIDs such as paracetamol, possesses mild antimicrobial properties. In a recent study, the MIC values of paracetamol against *K. pneumoniae* and *E. faecalis* strains were reported as 0.125 and 0.25 mg/mL, respectively (Aydın, 2019). Yadav et al. (2021) measured the comparative antimicrobial activity spectrum of NSAIDs in 37 genera 499 species and found that paracetamol inhibited

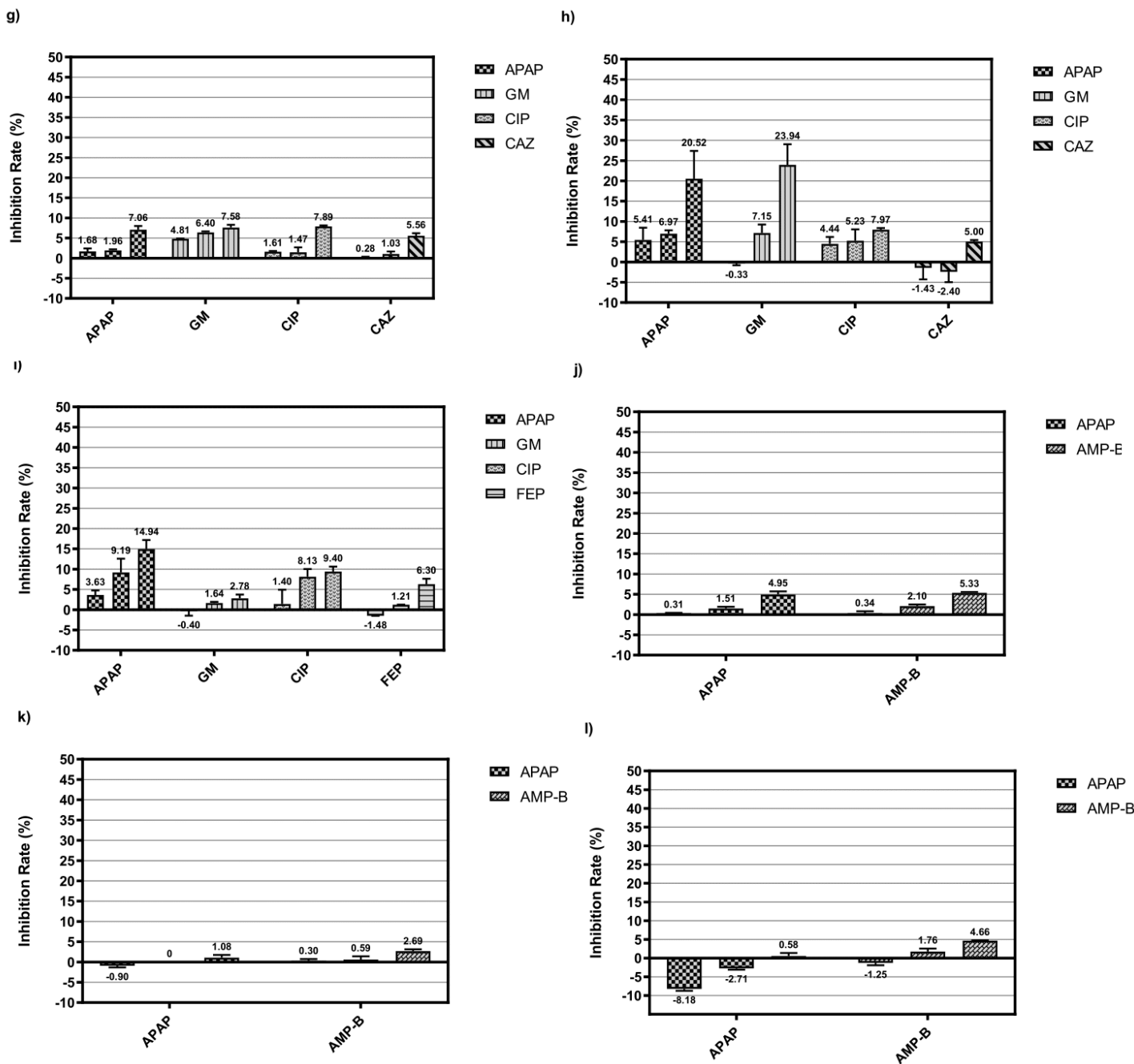
most of the strains tested at a concentration of <12.8 mg/mL. In another study made by Grecka and Szweda (2021), it was determined that the MIC and MBC values of paracetamol against *S. aureus* ATCC 29213 strain were >8,000 µg/mL. Also, other researchers found that the paracetamol MIC against *S. aureus* ATCC 29213, was 6,250 µg/mL (Öztürk et al., 2021). In a study in which the active ingredient of thirteen FDA approved non-antimicrobial drugs was tested by microdilution method, the MIC value of paracetamol against *A. baumannii* strain was found to be 256 µg/mL (Ugurel & Turgut-Balik, 2023). Król et al. (2018), investigated the anti-candidal activity of some NSAIDs and the MIC value of paracetamol was found >5 mmol/l against three of *Candida* species, which were also used in this study (Król et al., 2018).

In present study, combinations of paracetamol with antimicrobial agents against standard bacterial and fungal strains were also investigated. According to the results, cefepime and paracetamol combinations against *E. coli* and *A. xylosoxidans* strains; gentamicin and paracetamol combinations against *K. pneumoniae* and *A. baumannii* strains, showed synergistic effect. No antagonist effect was observed against any strain studied.

Combinations of NSAIDs with various antibiotics, antifungals and other antimicrobial agents have also been studied by different researchers. Altaf et al. (2019) suggested that flunixin meglumine can be used in combination with oxytetracycline against methicillin-resistant *S. aureus* infection; therefore, resistance can be successfully managed by combining antibiotics with NSAIDs (Altaf et al., 2019). In another study a 4-fold reduction in gentamicin MIC was demonstrated when combined with paracetamol (Öztürk et al., 2021). In a recent study, the combination of propolis with antipyretic agents such as paracetamol was proven to have a synergistic effect (Grecka & Szweda, 2021). Similarly, other researchers have determined that synergistic effects may be observed when NSAIDs are used in combination with various antimicrobial agents (Chan et al., 2017; Gil et al., 2020).

However, according to the literature review, there were no studies investigating the combination of paracetamol and antimicrobial agents against *P. mirabilis*, *A. xylosoxidans*, *K. pneumoniae* and *Candida spp.* Therefore, these results are the first findings on the combination of paracetamol and antimicrobial agents against these microorganisms.

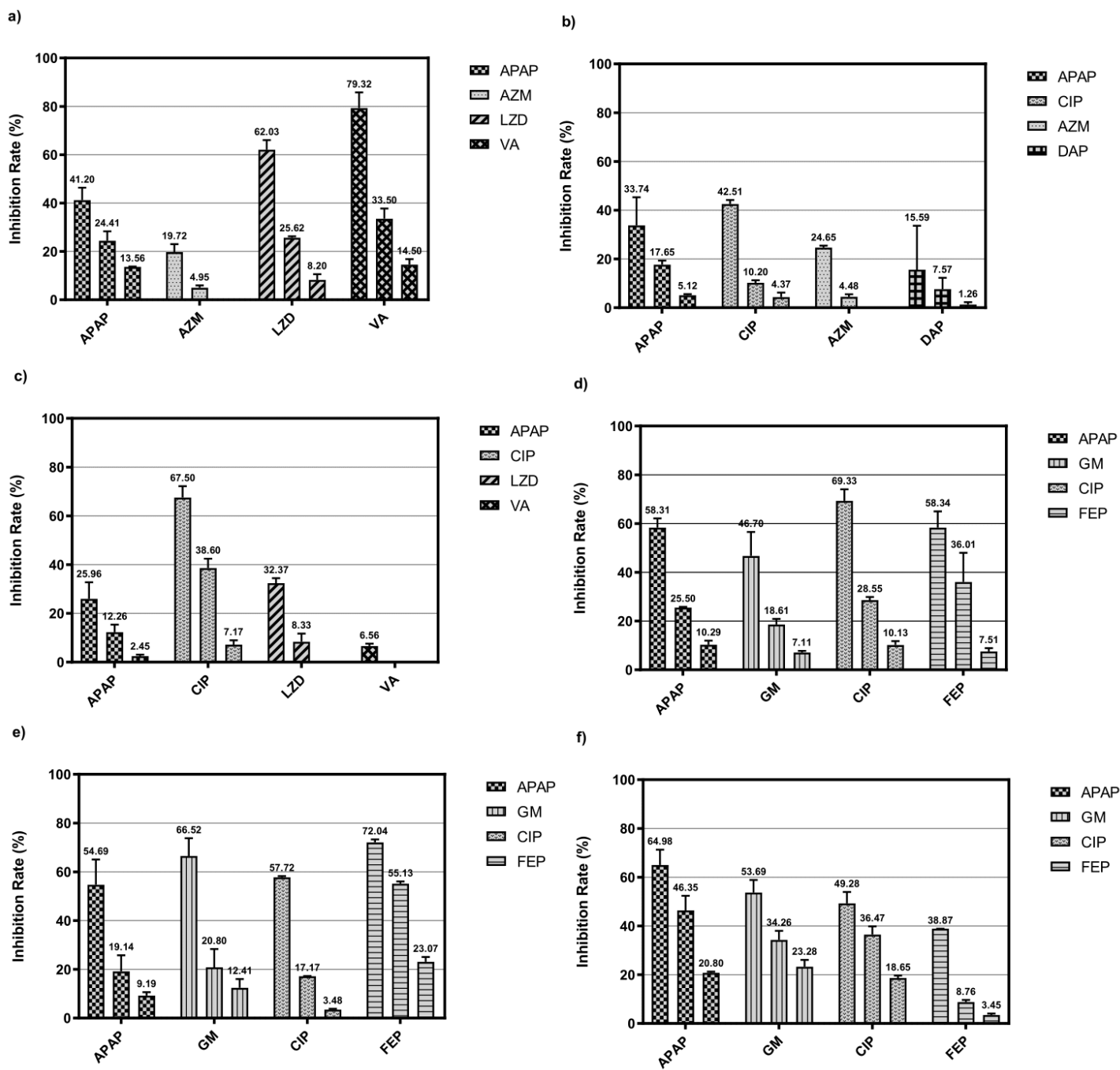




**Figure 1.** Effects of paracetamol and antimicrobial agents on the adhesion of microorganisms at 1/10xMIC. The bars show the results of the inhibition rates (%) at 1, 2 and 4 h for bacteria and 2, 4 and 6 h for *Candida spp.*, respectively.

a) *Staphylococcus aureus* b) *Staphylococcus epidermidis* c) *Enterococcus faecalis* d) *Escherichia coli* e) *Klebsiella pneumoniae* f) *Proteus mirabilis* g) *Pseudomonas aeruginosa* h) *Acinetobacter baumannii* i) *Achromobacter xylosoxidans* j) *Candida albicans* k) *Candida parapsilosis* l) *Candida tropicalis*

APAP: Paracetamol, AZM: Azithromycin; DAP: Daptomycin; GM: Gentamicin; LZD: Linezolid; FEP: Cefepime; CAZ: Ceftazidime; CIP: Ciprofloxacin; VA: Vancomycin; AMP-B: Amphotericin B



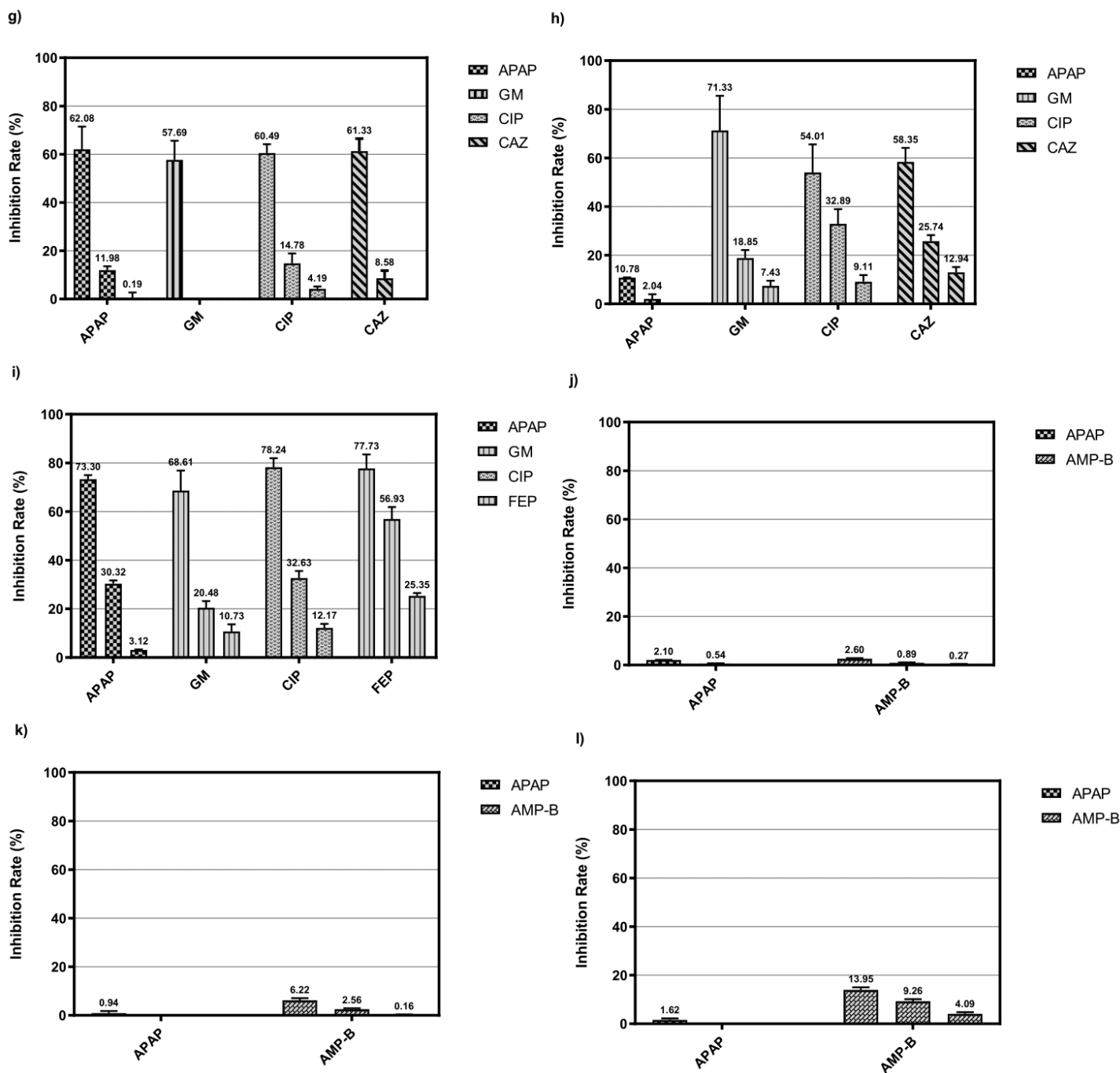


Figure 2. Effects of paracetamol and antimicrobial agents at different concentrations on biofilm formation of microorganisms after 24 h incubation. The bars show the results of the inhibition rates (%) at 1xMIC, 1/10xMIC, 1/100xMIC values, respectively.

a) *Staphylococcus aureus* b) *Staphylococcus epidermidis* c) *Enterococcus faecalis* d) *Escherichia coli* e) *Klebsiella pneumoniae* f) *Proteus mirabilis* g) *Pseudomonas aeruginosa* h) *Acinetobacter baumannii* i) *Achromobacter xylosoxidans* j) *Candida albicans* k) *Candida parapsilosis* l) *Candida tropicalis*

APAP: Paracetamol, AZM: Azithromycin; DAP: Daptomycin; GM: Gentamicin; LZD: Linezolid; FEP: Cefepime; CAZ: Ceftazidime; CIP: Ciprofloxacin; VA: Vancomycin; AMP-B: Amphotericin B



Biofilms are defined as communities formed by microorganisms adhering to each other or to a surface and are formed by various bacterial and fungal species. Resistance to antimicrobial agents in biofilms is usually the result of limited penetration, expression of various resistance genes, and altered reproductive and metabolic activities of the cells; therefore, cells in biofilms are more resistant to antimicrobial agents than planktonic cells (Ciofu et al., 2015). Due to antimicrobial resistance in biofilms, most persistent chronic infections are known to be associated with biofilms and they are now recognized as one of the main targets for the development of antimicrobial agents (Li & Lee, 2017).

In the third part of the present study, the effects of paracetamol and antimicrobial agents against adhesion and biofilm formation of various standard gram-negative and gram-positive bacteria and *Candida* were investigated. According to the results, paracetamol inhibited adhesion, although to different degrees depending on the bacterium with the highest inhibitory effect against *A. baumannii*, *K. pneumoniae* and *A. xylosoxidans* with 20.5%, 14% and 14% inhibition ratios, respectively. Paracetamol was also found to be more effective at inhibiting biofilm formation of gram-negative bacteria (up to 73.3% inhibition rates) than gram-positive bacteria and yeast. However, amphotericin B was found to be more effective than paracetamol at both biofilm adhesion and formation inhibition against *Candida* species.

In order for microorganisms to form biofilms, they must first adhere strongly to a surface. After adhesion, the microorganisms that settle in that area multiply to reach a certain density, while on the other hand, they start to form biofilm, and thus biofilm formation can be prevented by preventing adhesion.

Seleem et al. (2021) showed that paracetamol inhibited adhesion and biofilm formation by inhibiting virulence factors of *P. aeruginosa* PA01 and *A. baumannii*. Thus, according to this study, it was reported that paracetamol has anti-QS activity in addition to its analgesic effect and can be used together with antibiotics in the treatment of these infections (Seleem et al., 2021). Another study evaluated the effects of paracetamol exposure on *S. aureus* biofilm formation and reported that different doses of paracetamol had a limited effect on the growth of *S. aureus* planktonic cells and did not significantly change the metabolic rate, but modulated polysaccharide expression during biofilm formation (Sultan et al., 2021). Abidi et al. (2019) investigated the antibiofilm activity of acetylsalicylic acid, mefenamic acid and paracetamol on *P. aeruginosa* and *S. epidermidis* strains and reported that paracetamol was the most effective agent in reducing biofilm formation which was reduced biofilm formation of *P. aeruginosa*

by 40% and *S. epidermidis* by 25% (Abidi et al., 2019) In a recent *in vitro* study showed that paracetamol and ibuprofen have antibiofilm effect with concentration-dependent activity where the concentration/dose exposed is higher along with it providing a stronger effect to inhibit the formation of biofilms and it has been proven that paracetamol (1000 mg), inhibited *P. aeruginosa* biofilm formation up to 68.15% (Sihotang et al., 2022).

According to the literature review, the limited and small number of articles have investigated the antibiofilm effect of paracetamol. The results of our study and those of previous studies have shown that NSAIDs, including paracetamol, can inhibit biofilm formation by reducing bacterial adhesion. However, to date there are no data in the literature that investigated the inhibition of biofilm adhesion and formation by paracetamol against *E. faecalis*; *E. coli*; *K. pneumoniae*; *P. mirabilis*; *A. xylosoxidans* and *Candida spp.* The findings of present study are very important as they may provide new approaches to prevent the formation of biofilm infections, which are very difficult to treat.

## CONCLUSION

In conclusion, the antimicrobial and antibiofilm activities of paracetamol, one of the most widely used analgesic and antipyretic agents in clinical use for about 70 years, was investigated. It has been found that its combination with cefepim and gentamicin has been found to be synergistic against various bacteria. Furthermore, according to the results, paracetamol inhibited adhesion, although to different degrees depending on the bacterium. Thus, it has been shown that paracetamol can be used as an adjuvant in bacterial and fungal infections to inhibit biofilm-associated infections and reduce antimicrobial resistance. In addition, in the development of a topical antimicrobial formulation to reduce the use of antibiotics, paracetamol is one of the first choice NSAIDs because of its safe profile. However, further investigation of the underlying mechanisms with *in vivo* studies is also required to develop a safe drug combination.



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