



# Antibacterial activity of curcumin – a natural phenylpropanoid dimer from the rhizomes of *Curcuma longa* L. and its synergy with antibiotics

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## Abstract

**Introduction and Objective.** Curcumin is a natural substance extracted from turmeric that has broad-spectrum antibacterial properties. The aim of the study was to assess the antibacterial activity of curcumin and its interactions with selected antibiotics against drug-sensitive and multidrug-resistant isolates of emerging bacterial pathogens frequently found in healthcare settings.

**Materials and method.** Antibacterial activity of curcumin was assessed by MIC and MBC values against a total of 30 drug-sensitive and drug-resistant bacterial strains. Curcumin synergy with antibiotics was identified by the checkerboard method.

**Results.** Curcumin has much greater antibacterial activity against Gram (+) bacteria than against Gram (-) organisms. Curcumin showed the best effectiveness against *S. aureus*, regardless of antibiotic resistance. Since the combination of curcumin with antibacterial drugs inhibited *E. coli*, *E. faecalis* and *S. aureus*, in most cases showing synergistic relationship, the potential use of such combinations can be used to develop an alternative drug in the treatment of selected human pathogens. However, the combination of curcumin with antibiotics did not result in a satisfactory effect in relation to *K. pneumoniae* MBL and KPC strains as well as *P. aeruginosa*, showing indifferent or antagonistic interactions.

**Conclusions.** As shown by the *in vitro* data obtained in this study, curcumin has a broad spectrum of antibacterial activity, including emerging and multi-drug-resistant strains. However, the antibacterial effect seems to be bacteria-dependent, and can differ within bacterial species.

## Key words

curcumin, synergy, multidrug-resistant bacteria, emerging bacterial pathogens

## INTRODUCTION

Turmeric (*Curcuma longa*) is a perennial plant of the ginger family and includes roughly 104 plant species. The plant is often used as a spice in oriental dishes, as a colouring agent in both food and industry, and as a medicinal agent. The plant originates from India but is cultivated in many tropical countries, and has long been used in the herbal and dye industries as a spice, or as a cosmetic agent for making lipsticks and oriental perfumes [1, 2]. Turmeric has been used in Indian medicine by local healers and herbalists for millennia, and was often attributed with mystical, magical or religious properties. It was often used as a remedy for digestive and respiratory ailments or treating wounds, and was most often applied in the form of infusions, essential oils or compresses made from the rhizome [1]. Thanks to its effective action, the use of turmeric has spread to surrounding regions such as China and the Middle East, where it has found its unique applications [1, 2]. Due to its many uses, it was a highly valued commodity, which also contributed to its immense popularity and eventually caused its spread worldwide. From the medical point of view, the most valuable

part in turmeric is its rhizome, which contains about 70% starch, 3–5% curcuminoids – especially curcumin, volatile oils (e.g. germacron, elmenone, zingiberene, turmerone, curlon) and resins – approximately 5% [1, 2, 3]. As shown by studies on the substances that have a key influence on the properties of turmeric, are curcuminoids, which come in three types and approximately 77% of all curcuminoids are curcumin, 17% are demethoxycurcumin and 3% are bisdemethoxycurcumin, of which curcumin is the most active [1]. For this reason, most current research on this plant focuses on this compound. In 1815, curcumin was first isolated from turmeric, but it was not until 1910 that Polish scientists J. Miłobędzka, K. Kostanecki and W. Lampe established its spatial structure, which has a significant impact on the therapeutic effect of turmeric [4].

Curcumin is an organic compound composed of two feruloyl residues linked by a carbon bond and is classified as a polyphenolic compound. It is known as diferuloylmethane, IUPAC name – (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, chemical formula – C<sub>21</sub>H<sub>20</sub>O<sub>6</sub>, molecular weight – 368.38. It is obtained by extraction from turmeric rhizome using methods such as a Soxhlet apparatus, ultrasound, microwave and supercritical carbon dioxide, and subsequent separation on a chromatographic column [1, 5]. It has been attributed with a number of antibacterial, antidiabetic, anti-inflammatory, anticancer and antioxidant

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properties [3, 6, 7]. Despite many studies on the effects of curcumin, the mechanism responsible for its antibacterial and antifungal properties has not been established with a high degree of certainty. Various researchers claim that it blocks DNA replication, alters gene expression in the cell, damages the cell membrane, and decreases the overall mobility of microorganisms [3, 8]. Some studies also indicate that curcumin may interfere with the polymerisation of protofilaments of the FtsZ protein, which is a prokaryotic homologue of tubulin that forms the protofilaments of microtubules that act as the cytoskeleton providing the cell with a stable shape [9]. Other studies have observed that curcumin stimulates apoptosis in *Escherichia coli*, and different studies have shown that curcumin and its new derivatives, such as gallic curcumin and Cu curcumin, have remarkable antiviral effects on HSV-1 in cell culture [10, 11]. With these broad properties, curcumin can be used as an ingredient in many remedies against bacterial, fungal and viral infections. New mechanisms that may be responsible for these properties are still being discovered.

One of the biggest challenges of modern medicine is the urgent issue of combating multi-drug resistant bacteria. Unfortunately, the number of new antibiotics is not sufficient to meet future demands, therefore it is extremely promising to strengthen the effect of already used antibiotics by their cooperation with herbal agents [12]. These substances have recently gained considerable popularity and may provide a solution to the growing problem of multidrug resistant bacteria.

This study was aimed at assessing the antibacterial activity of curcumin and its interactions with selected antibiotics against drug-sensitive and multidrug-resistant isolates of emerging bacterial pathogens that are frequently found in healthcare settings.

## MATERIALS AND METHOD

**Turmeric root juice and curcumin.** In order to obtain the turmeric extract, about 100 g of the washed root was crushed with mortar and pestle. The extract was then sieve through a fine mesh cloth and sterilized using a membrane filter (0.45 µm). This extract was considered as the 100% concentration of the extract.

Curcumin from *Curcuma longa* (C1386 Sigma-Aldrich) was dissolved in DMSO dimethylsulfoxide (Pan Biotech) at a starting concentration of 200 mg/ml.

**Bacterial strains.** In this study, a total of 30 drug-sensitive and drug-resistant bacterial strains (11 multidrug-resistant strains and 19 drug-sensitive strains) were used. All bacterial strains were grown on triptic soy agar (TSA) at 37 °C. Table 1 shows the bacterial species, strain origin and resistance phenotypes found in the microorganisms used in the experiments.

**Determination of the minimum inhibitory concentration (MIC) of curcumin.** The MIC determination was performed according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using the modified broth dilution method in a 96-well microtiter plate format, as previously described [13]. Bacterial colonies from the MHA plates

**Table 1.** Bacterial strains used in the study

No.	Bacterial strain	Origin of the strain	Phenotype of antibiotic resistance
1.	<i>Escherichia coli</i>	ATCC 35218	
2.	<i>Escherichia coli</i>	ATCC 25922	
3.	<i>Escherichia coli</i>	MMC 41	
4.	<i>Escherichia coli</i>	MMC 122	
5.	<i>Escherichia coli</i>	MMC 124	
6.	<i>Escherichia coli</i>	MMC 237	
7.	<i>Staphylococcus aureus</i>	ATCC 29213	
8.	<i>Staphylococcus aureus</i>	NCTC 12493	MRSA
9.	<i>Staphylococcus aureus</i>	MMC 21	
10.	<i>Staphylococcus aureus</i>	MMC 89	MLS <sub>B</sub> , MRSA
11.	<i>Staphylococcus aureus</i>	MMC 61	MLS <sub>B</sub> , MSSA
12.	<i>Staphylococcus aureus</i>	MMC 41	MRSA
13.	<i>Klebsiella pneumoniae</i>	NCTC 13438	KPC (+)
14.	<i>Klebsiella pneumoniae</i>	NCTC 13440	MBL (+)
15.	<i>Klebsiella pneumoniae</i>	NCTC 13442	OXA-48 (+)
16.	<i>Klebsiella pneumoniae</i>	ATCC 700603	ESBL (+)
17.	<i>Klebsiella pneumoniae</i>	MMC 374	
18.	<i>Klebsiella pneumoniae</i>	MMC 314	NDM-1 (+) OXA-48 (+)
19.	<i>Klebsiella pneumoniae</i>	MMC 307	
20.	<i>Pseudomonas aeruginosa</i>	ATCC 27853	
21.	<i>Pseudomonas aeruginosa</i>	MMC 256	
22.	<i>Pseudomonas aeruginosa</i>	MMC 258	
23.	<i>Pseudomonas aeruginosa</i>	MMC 223	
24.	<i>Pseudomonas aeruginosa</i>	MMC 55	
25.	<i>Pseudomonas aeruginosa</i>	ATCC 9027	
26.	<i>Enterococcus gallinarum</i>	MMC 43	HLAR, VRE, ARE
27.	<i>Enterococcus faecalis</i>	MMC 44	HLAR, VRE
28.	<i>Enterococcus faecalis</i>	ATCC 29212	
29.	<i>Enterobacter cloacea</i>	MMC 104	
30.	<i>Citrobacter koseri</i>	MMC 92	

MMC - strains from the collection of the Department of Medical Microbiology  
 MRSA - methicillin-resistant *Staphylococcus aureus*  
 MLS<sub>B</sub> - cross-resistance to macrolides, lincosamides, and streptogramin B  
 ESBL - extended-spectrum beta-lactamases  
 KPC - *Klebsiella pneumoniae* carbapenemase  
 MBL - metallo-β-lactamase  
 OXA-48 (+) - Oxacillinase-48  
 NDM-1 (+) - New Delhi metallo-β-lactamases  
 HLAR - high-level aminoglycoside resistance  
 VRE - vancomycin resistant *Enterococcus*  
 ARE - ampicillin resistant *Enterococcus*

incubated overnight at 37 °C were resuspended in sterile NaCl and adjusted to the 0.5 McFarland standard of approximately  $1.2 \times 10^8$  CFU/ml.

For broth microdilution, susceptibility panels in 96-well microtiter plates were prepared by adding 100 µl of Mueller-Hinton broth (MHB) and dispensing a solution of curcumin in DMSO at a concentration of 10 mg/ml for Gram (+) bacteria and a solution of 80 mg/ml for Gram (-) bacteria. Then, two-fold serial dilutions of the curcumin extract were made to achieve the final concentrations. The last two wells were left without curcumin as a control for bacterial growth in the broth. The procedure was repeated with 100 µl DMSO (curcumin diluent) and 100 µl gentamicin with a starting concentration of 60 mg/ml (positive control wells). Aliquots (1,000 µl) of each bacterial suspension were inoculated into

the wells of the microtiter plates. The 96-microwell plates were incubated at 37°C for 24 h. The MICs of the curcumin were recorded as the lowest concentration where no viability was observed in the wells of the 96-microwell plates after incubation for 24 h. After incubation, 50 µl of a 0.2 mg/ml aqueous solution of MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to all wells, and further incubated for 30 min at room temperature. MIC was defined as the lowest concentration in which no transformation of MTT was observed. All samples were tested in triplicate and the tests repeated twice.

**Determination of minimum bactericidal concentration (MBC) of curcumin.** To determine the MBC value of curcumin, 10 µl from each well of the plate were inoculated onto the MHA medium and incubated for 24 h. The lowest concentrations that showed no growth after 24 h gave the MBC value.

**Checkerboard method for the combination.** The antimicrobial effect of combinations consisting of curcumin with selected antibiotics was assessed with the checkerboard method. The antibiotics for the study were selected from the set of antibiotics recommended for the given types of bacteria according to the European Committee on Antimicrobial Susceptibility Testing EUCAST. The antimicrobial assays were performed with curcumin in combination with ampicillin, gentamycin and ciprofloxacin for Gram (+) bacteria and gentamycin, ciprofloxacin and piperacillin + tazobactam for Gram (-) organisms.

Serial dilutions of antibiotics to at least double the MIC were prepared according to the recommendations of EUCAST immediately prior to testing, and mixed in a 96-well plate containing varying concentrations of curcumin in CSMHB. Each well was inoculated with 100 µl of the bacterial inoculum of  $5 \times 10^5$  CFU/ml, and the plates were incubated at 35°C for 24h.

To validate the MIC, the fractional inhibitory concentration (FIC) was calculated. The following formulas were used to calculate the FIC index:

$$\begin{aligned} \text{FIC}_{\text{CUR}} &= (\text{MIC}_{\text{CUR}} \text{ in combination}) / (\text{MIC}_{\text{CUR}} \text{ alone}), \\ \text{FIC}_{\text{antibiotic}} &= (\text{MIC}_{\text{antibiotic}} \text{ in combination}) / \\ &\quad (\text{MIC}_{\text{antibiotic}} \text{ alone}), \\ \text{and the FIC index} &= \text{FIC}_{\text{CUR}} + \text{FIC}_{\text{antibiotic}}. \end{aligned}$$

The FIC indices were used to characterize the antibiotic interactions as follows:

**Synergy:** when the combination of compounds results in a FIC value of <0.5, it increases the inhibitory activity (decrease in MIC) of one or both compounds in comparison with the compounds alone.

**Additivity or indifference:** when the combination of compounds results in a FIC value of 0.5 – 4, there is no increase in inhibitory activity or a slight increase in inhibitory activity from the additive effect of both compounds combined.

**Antagonism:** when the combination of compounds results in a FIC value of >4, it increases the MIC or lowers the activity of the compounds (Pillai et al., 2013).

**Statistical analysis.** Each result is indicated as the mean value of at least three to five independent experiments ± the standard error of the mean (SEM). Significant difference was analyzed by Student's *t*-test. A *P*-value of 0.05 was used as the cut-off for statistical significance. Results were analyzed by means of Microsoft Excel programme packets.

## RESULTS

**Determination of MIC and MBC values of curcumin.** As indicated in Table 2, *Escherichia coli* strains showed MIC for curcumin in the range of 0.312 – 1.250 mg/ml (mean MIC 0.885 mg/ml), while MBC for these strains ranged from 1.25–5 mg/ml (mean MBC 3.54 mg/ml). The MIC for *Staphylococcus aureus* ranged from 0.039 – 0.078 mg/ml (mean MIC 0.046 mg/ml) and the MBC 0.039 – 0.625 mg/ml (mean MBC 0.19 mg/ml). For three strains of *Staphylococcus aureus*, the MIC value was equal to the MBC value. For the *K. pneumoniae* strains, the MIC for curcumin was in the range of 0.625 – 1.250 mg/ml (mean 1.04 mg/ml), while the MBC for these strains ranged from 2.5 mg/ml, up to above 20 mg/ml. For *Pseudomonas aeruginosa*, the MIC for curcumin was the highest of all species tested, ranging from 2.5 – 5 mg/ml (mean 3.75 mg/ml), while the MBC oscillated from 5 mg/ml to values above 20 mg/ml. For *Enterococcus gallinarum*, the MIC was 0.313 mg/ml and the MBC was higher than 5 mg/ml, while *E. faecalis* strains showed MICs ranging from 0.313 – 0.625 mg/ml (mean 0.469 mg/ml), and MBCs higher than 5 mg/ml. The MIC for *Enterobacter cloacea* was 5 mg/ml and MBC was above 20 mg/ml, for *Citrobacter koseri*, MIC of 1.250 mg/ml and MBC equal to 20 mg/ml was observed.

The lowest mean MIC and MBC of curcumin was recorded for *S. aureus* strains, while the highest MIC was shown by *E. cloacea* and three strains of *P. aeruginosa*. The highest MBC values (above 20 mg/ml) were found in five out of 29 strains tested including three strains of *Pseudomonas aeruginosa* and one strain of *Klebsiella pneumoniae* and *Enterobacter cloacea*. The MIC values of curcumin for Gram (+) bacteria (*Staphylococcus* and *Enterococcus*) were lower than for Gram (-) bacilli, the medium MIC for cocci being 0.092 mg/ml and for Gram-negative bacilli 0.6 mg/ml. While the lowest MBC values of curcumin were observed for *Staphylococcus aureus* 0.039 mg/ml, for the other species the MBC values ranged from 0.312 mg/ml – > 20 mg/ml.

Regarding the tested multidrug-resistant strains, the mean MIC for MRSA strains was 0.065 mg/ml and a similarly low MIC was recorded for staphylococci with MLSB phenotype, 0.039 mg/ml. The MIC of curcumin for enterococci showed similar values for HLAR and VRE strains (mean MIC 0.469 mg/ml) as the MIC obtained for the antibiotic-sensitive strain (0.313 mg/ml). As for *Klebsiella pneumoniae* producing KPC and OXA-48 type carbapenemases, the MIC for curcumin was 0.625 mg/ml. A higher MIC of 1.250 mg/ml was observed for *Klebsiella pneumoniae* strain producing MBL carbapenemases, and for *Klebsiella pneumoniae* strain simultaneously producing NDM-1 and OXA-48 type carbapenemases.

The study showed no effect of DMSO on the results obtained, and control of the MIC determination method with gentamicin gave results consistent with those expected for the reference strains.

**Table 2.** MIC and MBC values of curcumin for the tested bacterial strains

No.	Bacterial species	Collection and phenotype of resistance	Curcumin MIC (mg/ml)	Curcumin MBC (mg/ml)	DMSO MIC %	GM MIC (µg/ml)
1.	<i>Escherichia coli</i>	ATCC 35218	0.625	2.5	12.5	0.468 (0.25-1) <sup>^</sup>
2.		ATCC 25922	1.250	5	12.5	0.9375
3.		MMC 41	0.625	2.5	12.5	1.875
4.		MMC 122; ESBL (-)	1.250	5	12.5	0.938
5.		MMC 124; ESBL (-)	1.250	5	12.5	0.468
6.		MMC 237	0.312	1.25	12.5	7.5
7.	<i>Staphylococcus aureus</i>	ATCC 29213; MSSA	0.039	0.039	25	0.468 (0.25-0.5) <sup>^</sup>
8.		NCTC 12493; MRSA	0.039	0.039	25	0.938
9.		MMC 21; MSSA	0.039	0.039	25	>15
10.		MMC89; MLSB, MRSA	0.078	0.625	25	0.058
11.		MMC 61; MLSB, MSSA	0.039	0.078	25	0.234
12.		MMC 41; MRSA	0.078	0.312	25	0.468
13.	<i>Klebsiella pneumoniae</i>	NCTC 13438; KPC (+)	0.625	>5	12.5	3.75
14.		NCTC 13440; MBL (+)	1.250	>10	12.5	3.75
15.		NCTC 13442; OXA-48 (+)	0.625	2.5	12.5	0.468
16.		MMC 374	1.250	10	12.5	0.234
17.		MMC 314; NDM-1 (+) OXA-48 (+)	1.250	20	12.5	>15
18.		MMC 307	1.250	>20	12.5	0.468
19.	<i>Pseudomonas aeruginosa</i>	ATCC 27853	5	>20	6.25	1.875 (0.5-2) <sup>^</sup>
20.		MMC 256	2.5	20	6.25	1.875
21.		MMC 258	5	20	6.25	3.75
22.		MMC 223	2.5	5	6.25	>15
23.		MMC 55	2.5	>20	6.25	>15
24.		ATCC 9027	5	>20	12.5	1.875
25.	<i>Enterococcus gallinarum</i>	MMC 43 AMR HLAR, VRE,	0.313	>5	6.25	>15
26.		MMC 44; HLAR, VRE	0.625	>5	6.25	>15
27.	<i>Enterococcus faecalis</i>	ATCC 29212	0.313	>5	6.25	8 (4-16) <sup>^</sup>
28.	<i>Enterobacter cloacae</i>	MMC 104	5	>20	12.5	0.938
29.	<i>Citrobacter koseri</i>	MMC 92	1.250	20	12.5	0.468

<sup>^</sup>range of gentamicin reference MIC values for reference strains. Control: MIC DMSO and MIC gentamicin.

### Interaction between curcumin and antibiotics assessed by the checkerboard method.

Based on the FIC index, the antibacterial activities of  $\beta$ -lactam antibiotic – piperacillin with tazobactam were improved in the presence of curcumin against both tested *Escherichia coli* strains. No enhancing effect on the aminoglycoside and fluoroquinolone was observed, and both strains tested showed an antagonistic effect with gentamicin. In relation to the other tested Gram (-) bacteria (*K. pneumoniae* KPC and *P. aeruginosa*), antibiotics exhibited indifferent interactions in combination with curcumin (Tab. 3).

At the concentration tested, curcumin significantly improved antibiotic efficacy against *Enterococcus faecalis* when combined with ampicillin and gentamicin, and also significantly improved antibiotic efficacy against multidrug-resistant *S. aureus* when combined with gentamycin and ciprofloxacin. However, at the same time, combination effect of the antibiotics with curcumin against fully susceptible strain exhibited indifferent or antagonistic interactions.

**Table 3.** Antimicrobial effect of combinations consisting of curcumin (CUR) and selected antibiotics against the tested bacterial strains assessed with the checkerboard method

Bacterial strain	Agent	Ratio	$\Sigma$ FIC	Activity
<i>S. aureus</i> ATCC 29213	CUR-GM	0.02:2	2.02	I
	CUR-CIP	0.02:4	4.02	A
<i>S. aureus</i> MMC 89; MLSB (+), MRSA(+)	CUR-GM	0.0003:0.4	0.4	S
	CUR-CIP	0.001:0.4	0.4	S
<i>E. faecalis</i> ATCC 29212	CUR-GM	0.009:0.37	0.37	S
	CUR-AMP	0.012:0.44	0.44	S
<i>K. pneumoniae</i> NCTC 13440; (MBL+)	CUR-GM	0.001:0.33	0.33	S
	CUR-CIP	0.001:4	4	A
	CUR-TZP	0.0012:1.2	1.2	I
<i>K. pneumoniae</i> NCTC 13438; KPC (+)	CUR-GM	0.005:0.8	0.8	I
	CUR-CIP	0.002:0.99	1	I
	CUR-TZP	0.003:1.08	1	I
<i>E. coli</i> MMC 122	CUR-GM	0.003:4.3	4.3	A
	CUR-CIP	0.007:0.49	0.5	S
	CUR-TZP	0.004:0.3	0.3	S
<i>E. coli</i> ATCC 35218	CUR-GM	0.003:4.8	4.8	A
	CUR-CIP	0.008:0.47	0.47	I
	CUR-TZP	0.004:0.25	0.25	S
<i>P. aeruginosa</i> ATCC 27853	CUR-GM	0.0002:1	1	I
	CUR-CIP	0.003:1	1	I
	CUR-TZP	0.005:1	1	I

## DISCUSSION

The rapid emergence of multidrug-resistant bacteria and the paucity of effective antibiotics against them call for extensive research to identify more drugs to battle such life-threatening infections. Therefore, the use of herbal extracts as a source of therapeutic agents is receiving considerable attention nowadays in global health debates [15].

Recently, turmeric has been gaining the interest of researchers worldwide due to its versatile properties that make it an attractive substance from both a medical and consumer point of view. Among the components of turmeric, curcumin is the most important fraction, which is responsible for its antimicrobial activities.

It is now thought that curcumin's activity on the bacterial cell can be attributed mainly to the damage to the cell wall or cell membrane, interference on cellular processes by inhibiting bacterial DNA replication and altering gene expression [16, 17]. Moreover, it inhibits the bacterial quorum sensing process that regulates bacterial cell density and behaviour [16], and induces significant production of ROS, suggesting that oxidative stress may be the mechanism of antibacterial action of curcumin [16, 17]. Therefore, an important advantage of curcumin contained in turmeric extract over most antibiotics is that it affects multiple targets in the bacterial cell, making curcumin an interesting option for the elimination of antibiotic-resistant bacteria [16].

Previous studies have shown different antibacterial effects of curcumin depending on the strain of bacteria it affects [17, 18, 19]. However, insufficient data exist on its effects against bacterial clinical isolates and multidrug-resistant strains. In view of the imperative requirement of new anti-bacterial drugs, this study reports the activity of turmeric derivatives, with curcumin being the line of focus, against a wide panel of bacterial clinical isolates, including the most emerging pathogens.

Consistent with previous reports, curcumin showed a significantly higher activity against Gram (+) bacteria than Gram (-) bacteria [17, 19]. The highest activity of curcumin in the disc diffusion method, as well as in the broth dilution method, was observed against staphylococci. The zones of inhibition changed with the concentration of curcumin and ranged from 11 mm at a curcumin concentration of 200 mg/ml to 6.5 mm at a concentration of 0.1953 mg/ml. It should be noted that curcumin showed similar activity against the MRSA strain and the fully susceptible *Staphylococcus aureus* strain, suggesting that the sensitivity towards curcumin treatment is not altered by the multidrug resistance machinery in *S. aureus* [20].

The obtained results show the lower activity of curcumin against Gram (-) bacteria, when compared with the Gram (+) cocci (staphylococci and enterococci), with the highest recorded MIC value observed for *Pseudomonas aeruginosa* (5 mg/ml). This regularity has also been observed by others. Adamczak et al. observed the inhibitory effect of curcumin on the growth of *S. aureus* isolates at the concentrations of 0.250 mg/ml, whereas most of the Gram (-) bacterial strains tested showed a weak sensitivity to curcumin, reaching the MIC value up to 5 mg/ml [17].

The stronger effect of curcumin on Gram (+) bacteria than on Gram (-) bacteria is thought to be related to the cell wall composition. The cell wall of Gram (+) bacteria contains mainly peptidoglycan and hydrophobic molecules

that can pass through their cell wall easier. However, the complex composition of the outer membrane of Gram (-) bacteria forms a barrier for penetration of most hydrophobic molecules and antimicrobial agents such as  $\beta$ -lactams, quinolones and colistin [17, 21].

Today, the emergence of MDR bacteria, such as *S. aureus* MRSA and highly resistant *Enterobacteriaceae* carrying ESBL, MBL, KPC, NDM-1, OXA-48 resistance mechanisms, cause serious health problems, as broad-spectrum antimicrobial resistance significantly limits effective therapeutic options. The present study demonstrated high activity of curcumin against staphylococci with MRSA and MLSB resistance phenotypes, and showed similar MIC values at the mean level of 0.046 mg/ml. This fact clearly indicates that any antibiotic-resistance mechanism represented by these MDR bacteria seemingly has no impact on susceptibility to curcumin, and gives a good prospect for the use of curcumin in the treatment of infections caused by antibiotic-resistant staphylococci.

Broad spectrum antimicrobial resistance in carbapenemase-producing *Klebsiella pneumoniae* isolates significantly limits effective therapeutic options as they are resistant to all currently available antimicrobial agents [22]. Therefore, this study evaluated an alternative approach to treat infections caused by hyperepidemic strains, such as KPC, NDM-1, OXA-48 and MBL, examining how they would be affected by the use of curcumin. The result obtained for *Klebsiella pneumoniae* NDM and OXA-48 strains require special mention as they are resistant to all currently available antibiotics, and the mortality rate in the case of infections caused by these variants reaches widely from 22% – 72% [23, 24]. The present study demonstrated the antibacterial activity of curcumin at the concentration of 0.625 mg/ml on KPC and OXA-48 strains, and on the strain that simultaneously produce carbapenemases NDM-1 and OXA-48, as shown by the MIC 1.250 mg/ml of curcumin. Even though these concentrations are higher than those observed in Gram (+) bacteria, the fact gives real hope for the use of curcumin as a substance supporting the therapy of infections with such multidrug-resistant strains, however, this requires further studies.

It is important to mention the research carried out by Shi M. et al. By using virtual GPU-accelerated quantum experiments (GPU methods), the authors established that the keto forms of curcumin, catechin, menthol and ferulic acid, could be considered as inhibitors of the NDM-1-type of resistance [23]. This shows that curcumin could be a breakthrough in the treatment of *Klebsiella pneumoniae* New Delhi infected patients. Additional studies should be conducted to investigate the impact of administering different forms of curcumin to patients with gastrointestinal carriage of *Klebsiella pneumoniae* KPC or NDM-1 strains. It is possible that its administration could reduce the duration of carriage which would result in a significant decrease in the epidemic risk.

Of all the tested bacterial strains, *Pseudomonas aeruginosa* reference strains, as well as clinical isolates, exhibited relative curcumin resistance. Using curcumin on *P. aeruginosa*, MICs were very high, ranging from 2.5 mg/ml to 5 mg/ml. Previous research on the effects of curcumin on *P. aeruginosa* have shown that different strains of this bacterium react differently to this natural substance, and their resistance is mostly related to the composition and structures of their cell membranes [16, 17, 21, 25].

Based on its broad-spectrum antibacterial activity, using curcumin in combination with other existing drugs to potentiate their antimicrobial activity seems to be a promising approach. The application of the combination of herbal drugs with antibiotics has already been suggested, especially for antibiotic-resistant strains [16, 21, 26, 27]. Successful combination of the antibiotic with curcumin could reduce the dose of antibiotics and the compounds, by interacting with different targets in the bacterial cell can overcome its resistance mechanisms [21].

The present study investigated the potential of using curcumin as an adjunct to antibiotic therapy for synergistic association against selected Gram (+) and Gram (-) drug sensitive and multidrug-resistant pathogens. The antibacterial effect of the combination of curcumin with  $\beta$ -lactams and aminoglycosides significantly improved antibiotic efficacy against *Enterococcus faecalis*, which indicates a positive association between the two tested antimicrobial agents. The application of the combination of curcumin with anti-staphylococcal antibiotics on *S. aureus* MSSA and MLSB + MRSA strains differed depending on the strain and antibiotics susceptibility profiles. At the concentration tested, curcumin improved antibiotic efficacy against multidrug-resistant *S. aureus* when combined with gentamycin and ciprofloxacin, but at the same time, the combination effect of the antibiotics with curcumin against fully susceptible strain exhibited indifferent or antagonistic interactions.

Based on the reported findings, the antibacterial activity of antibiotics in the presence of curcumin is dependent on the resistance mechanism of the specific strain and is relatively nonspecific. As suggested by Mun et al., bacterial cell membrane permeability may be partly responsible in regulation of the antibacterial activity of curcumin in MRSA. The same authors found that the expression of penicillin binding protein 2a, which confers cross-resistance to most  $\beta$ -lactams, was downregulated in MRSA upon curcumin treatment [28].

In relation to the tested Gram (-) bacteria, the majority of antibiotics exhibited indifferent interactions in combination with curcumin, with exception of *E. coli*. In this study, two tested *E. coli* strains showed increased susceptibility to  $\beta$ -lactams in the presence of curcumin, with synergy observed. However, no enhancing effect on the aminoglycosides and fluoroquinolones was observed, and both tested strains showed an antagonistic effect with gentamicin. This is in accordance with the work of Rangel-Castañeda et al. who observed an increase in bactericidal activity against *E. coli* when  $\beta$ -lactams were administered with curcumin [29].

In light of the search for new therapeutic options to combat the most dangerous multidrug-resistant bacteria, the combination of curcumin with antibiotics did not produce a satisfactory effect in relation to *K pneumoniae* MBL and KPC strains, as well as *P. aeruginosa*, showing indifferent or antagonistic interactions.

At the present time, the reason of these differences is not known; however, when curcumin as an antibacterial agent enhances the action of other drugs, it may play a supportive role to improve the cellular uptake of other bacteriostatic agents by inhibiting the efflux pump rather than being the main antibacterial agent [21]. Further studies are therefore still needed to explore the specific action and properties of curcumin and turmeric extract on bacterial cells.

## CONCLUSION

Overall, the cumulative findings showed that curcumin has a broad spectrum of antibacterial activity, including emerging and multi-drug-resistant strains. However, the antibacterial effect seems to be bacteria-dependent, and can differ within bacterial species. Curcumin has much greater antibacterial activity against Gram (+) bacteria than against Gram (-) organisms, possibly due to differences in the structure of the cell membrane and the cell wall, the components of which may affect its anti-bacterial efficacy. Curcumin showed the best effectiveness against *S. aureus*, regardless of antibiotic resistance. Hence, it has high potential as a promising anti-staphylococcal agent in the future. Since the combination of curcumin with antibacterial drugs inhibited *E. coli*, *E. faecalis* and *S. aureus*, in most cases showing a synergistic relationship, the potential use of such combinations can be used to develop an alternative drug in the treatment of selected human pathogens.

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