Original Article

# Antibacterial synergy of curcumin with antibiotics against biofilm producing clinical bacterial isolates


## Abstract

**Introduction:** The role of natural bioactive substances in treating infections has been rediscovered as bacterial resistance become common to most of the antibiotics. Curcumin is a bioactive substance from turmeric. Owing to antimicrobial properties, its prospect as an antibacterial agent is currently under focus.

**Materials and Methods:** We have evaluated the *in vitro* synergy of curcumin with antibiotics against sixty biofilm producing bacterial isolates. Congo red agar method was used to identify the biofilm producing isolates. Curcumin minimum inhibitory concentration (MIC) was determined by agar dilution method. Its antibiotic synergy was identified by the increase in disc diffusion zone size on Mueller-Hinton agar with 32 mg/L curcumin.

**Results:** The mean MICs of curcumin against Gram-positive and Gram-negative isolates were 126.9 mg/L and 117.4 mg/L, respectively. Maximum synergy was observed with ciprofloxacin among Gram-positive and amikacin, gentamicin, and cefepime among Gram-negative isolates.

**Conclusions:** Curcumin *per se* as well as in combination with other antibiotics has a demonstrable antibacterial action against biofilm producing bacterial isolates. It may have a beneficial role in supplementing antibiotic therapy.



**Key words:**
*Antibacterial synergy, antibiotic, Curcuma longa, curcumin*


## Introduction

With the injudicious and rampant use, the benefit of antibiotics as antimicrobial therapy is fading. Resistance to most antibiotics has surged significantly in the recent years. Moreover, the increasing use of medical devices in postoperative wards, Intensive Care Units, and chronic care and life support facilities drastically raised the frequency of device-associated infections. Medical biofilm accounts for a large majority of these infections. Biofilm accounts for 65% of health-associated infections globally.[1] Biofilm producing organisms secrete extracellular polysaccharides which form a hydrated matrix encompassing diverse microbial population.[2] As a result, the pathogens remain in a secluded niche within the host body protected from antimicrobial agents and molecules of host immune defense. Moreover, the polymicrobial ecosystem in biofilm favors poly-species interaction and spread of resistance determinants.[3] While several antibiotics fail to exert their antibacterial action as they bind to the oppositely charged biofilm polymer, antibiotics such as imipenem and gentamicin at sub- minimum inhibitory concentration (MIC) doses are found to stimulate biofilm production.[4] Consequently, there is a need for novel strategies to control the growing threat of biofilm-associated infections. The natural bioactive substances have shown prospect in treating these infections. Curcumin, a phytochemical derived from the rhizome of *Curcuma longa*, has antibacterial as well as antibiofilm activity. Curcumin was found to restore


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bacterial susceptibility to antibiotics by inhibiting its biofilm mode of growth and rendering it sensitive to antibiotics *in vitro*.[5] Recently, its antibacterial action is found to be synergistic with several antibiotics.[6,7] However, there is limited research data on this synergistic antibacterial action of curcumin, especially against biofilm producing organisms to support its benefits in biofilm-associated infections. We conducted this study to determine the *in vitro* synergy of curcumin with antibiotics against biofilm producing clinical bacterial isolates.

## Materials and Methods

In this prospective study, we screened a total of 400 bacterial isolates from various clinical samples (pus, aspirate, blood, endotracheal secretion, sputum, and urine) received in the Department of Microbiology of our tertiary care teaching hospital for biofilm formation. Antibiotic susceptibility test (AST) was carried out on Mueller-Hinton agar (MHA) by Kirby–Bauer disc diffusion method for biofilm producing isolates.

### Detection of biofilm

Congo red agar method was used for detection of biofilm is bacteria isolates. All strains were inoculated on brain heart infusion agar containing 5% sucrose and 0.08% Congo red and were incubated aerobically at 37°C for 48 h. Bacterial strains which produced black colonies with crystalline texture were considered as biofilm producer and were selected for detection of the antibacterial action of curcumin. In contrast, colonies which remained pink and had no crystalline texture were identified as nonbiofilm producer.

### Determination of curcumin minimum inhibitory concentration

Agar dilution method was used to determine curcumin MIC. A stock solution (128 mg/L) of curcumin (Himedia, Mumbai, India) was prepared in dimethyl sulfoxide. It was used for the preparation of MHA plates with increasing concentration of curcumin. Required volumes of curcumin stock solution were added to MHA in a molten state to achieve the final concentrations of 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 mg/L. The bacterial strains were spot inoculated on these plates and were incubated aerobically at 37°C for 48 h. The minimum concentration of curcumin which demonstrated complete inhibition of growth was noted as MIC for that isolate.

### Detection of synergistic antibacterial action of curcumin with antibiotics

We tested the synergistic antibacterial action of curcumin with antibiotics against Gram-positive and Gram-negative isolates by disc diffusion method using MHA with 32 mg/L curcumin. Penicillin (10 μg), erythromycin (15 μg), ciprofloxacin (5 μg), and vancomycin (30 μg) discs were used for Gram-positive strains, whereas Gram-negative isolates were tested against ampicillin (10 μg), ceftriaxone (30 μg), cefepime (30 μg), gentamicin (10 μg), amikacin (30 μg), imipenem (10 μg), and meropenem (10 μg). The size of the zone of inhibition (ZOI) and susceptibility results of these isolates in the presence of curcumin were recorded and were compared to their zone sizes and susceptibility results on plain MHA (i.e., in the absence of curcumin).

### Quality control

*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used for quality control in ASTs.

## Results

Out of 400 clinical isolates, 60 (45 Gram-negative and 15 Gram-positive) bacterial strains were identified as biofilm producer by Congo red agar method and were selected for evaluation of *in vitro* antibacterial activity of curcumin. The bacterial isolates include *E. coli* ($n$ = 23), *Klebsiella pneumoniae* ($n$ = 13), *Citrobacter freundii* ($n$ = 6), *Pseudomonas aeruginosa* ($n$ = 3), *S. aureus* ($n$ = 13), and *Enterococcus faecalis* ($n$ = 2). The mean curcumin MIC for Gram-positive and Gram-negative isolates were 126.9 mg/L and 117.4 mg/L, respectively. AST of these isolates was carried out on plain MHA and on MHA with 32 mg/L curcumin, and the results are detailed in Tables 1 and 2. The supplementation of curcumin resulted in an increase in ZOI, as well as, change in interpretation of AST for most isolates [Table 3]. Few isolates showed no change in ZOI for ampicillin ($n$ = 34), ceftriaxone ($n$ = 26), gentamicin ($n$ = 22), amikacin ($n$ = 9), cefepime ($n$ = 4), imipenem ($n$ = 1), and meropenem ($n$ = 1).

## Discussion

The use of the herbal product in infective illness is not uncommon in traditional medicine. Turmeric, the rhizome of an Indian medicinal plant *C. longa*, has been known for its anti-inflammatory, carminative, hepatoprotective,

**Table 1: Antibiotic susceptibility of Gram-negative isolates ($n$=45) before and after curcumin supplementation**

| | MHA without curcumin | | | MHA with curcumin (32 mg/L) | | |
|---|---|---|---|---|---|---|
| | Resistant (%) | Intermediate (%) | Sensitive (%) | Resistant (%) | Intermediate (%) | Sensitive (%) |
| Ampicillin | 45 (100) | - | - | 44 (97.8) | - | 1 (2.2) |
| Ceftriaxone | 33 (73.3) | 2 (4.4) | 10 (22.2) | 29 (64.4) | 1 (2.2) | 15 (33.3) |
| Cefepime | 4 (8.9) | 8 (17.8) | 33 (73.3) | 3 (6.7) | 1 (2.2) | 41 (91.1) |
| Gentamicin | 31 (68.9) | 12 (26.7) | 2 (4.4) | 21 (46.7) | 9 (20) | 15 (33.3) |
| Amikacin | 10 (22.2) | 17 (37.8) | 18 (40) | 6 (13.3) | 3 (6.7) | 36 (80) |
| Imipenem | 2 (4.4) | 7 (15.6) | 36 (80) | 1 (2.2) | 4 (8.9) | 40 (88.9) |
| Meropenem | 2 (4.4) | 2 (4.4) | 41 (91.1) | 2 (4.4) | - | 43 (95.6) |

MHA: Mueller-Hinton agar

**Table 2: Antibiotic susceptibility of Gram-positive isolates (*n*=15) before and after curcumin supplementation**

| | MHA without curcumin | | | MHA with curcumin (32 mg/L) | | |
|---|---|---|---|---|---|---|
| | Resistant (%) | Intermediate | Sensitive (%) | Resistant (%) | Intermediate | Sensitive (%) |
| Penicillin | 15 (100) | - | - | 11 (73.3) | - | 4 (26.7) |
| Erythromycin | 15 (100) | - | - | 13 (86.7) | - | 2 (13.3) |
| Ciprofloxacin | 15 (100) | - | - | 7 (46.7) | - | 8 (53.3) |
| Vancomycin | - | - | 15 (100) | - | - | 15 (100) |

MHA: Mueller-Hinton agar

**Table 3: Change in antibiotic susceptibility results in presence of curcumin**

| | Number of isolates with changed AST | AST changed from resistant to intermediate | AST changed from resistant to sensitive | AST changed from intermediate to sensitive |
|---|---|---|---|---|
| Gram-negative isolates (*n*=45) | | | | |
| Ampicillin | 1 | 0 | 1 | 0 |
| Ceftriaxone | 6 | 1 | 3 | 2 |
| Cefepime | 9 | 1 | 0 | 8 |
| Gentamicin | 16 | 3 | 7 | 6 |
| Amikacin | 18 | 0 | 4 | 14 |
| Imipenem | 5 | 1 | 0 | 4 |
| Meropenem | 2 | 0 | 0 | 2 |
| Gram-positive isolates (*n*=15) | | | | |
| Penicillin | 4 | 0 | 4 | 0 |
| Erythromycin | 2 | 0 | 2 | 0 |
| Ciprofloxacin | 8 | 0 | 8 | 0 |
| Vancomycin | 0 | 0 | 0 | 0 |

AST: Antibiotic susceptibility test

anticancer, antioxidant, and antilipidemic actions in Ayurveda and Unani.[8] The extract of its rhizome contains a mixture of phenolic diarylheptanoids which are known as curcumoids. Curcumin or diferuloylmethane is the major active constituent. Current research has rediscovered its medical importance. Curcumin has antibacterial action. Authors have reported membrane alteration by curcumin could be attributed to its bactericidal action.[9,10] Curcumin being amphipathic and lipophilic easily gets incorporated in bacterial membrane resulting in its disruption and leakage.[9] In contrast, the antibiofilm effect is the result of modulating of gene expression and inhibition of quorum sensing. It not only inhibit biofilm but also downregulate several quorum sensing-dependent virulence factors such as the production of alginate, swarming, and motility.[11-13]

In our study, the mean curcumin MICs were 126.9 mg/L and 117.4 mg/L against Gram-positive and Gram-negative biofilm producing isolates, respectively. Due to the use of diverse methods, different preparations of curcumin and testing against different species of organisms, curcumin MIC varies widely in published literature. Sasidharan *et al.* reported curcumin-1 MIC ranging between 125 and 1000 μg/mL against bacteria causing infectious diarrhea.[6] In another study, methicillin-resistant *Staphylococcus aureus* (MRSA) isolates had curcumin MIC ranged from 125 to 250 μg/ml.[14] Gunes *et al.* determined the curcumin MIC of ATCC strains of *P. aeruginosa*, *Bacillus subtilis*, methicillin-sensitive *S. aureus*, MRSA, *E. coli*, *E. faecalis,* and *K. pneumonia,* which were 175, 129, 219, 217, 163, 293, and 216 μg/ml, respectively.[15]

There is a lack of published data on the antibiotic synergy of curcumin. The mechanism is yet to be confirmed. However, it is likely that inhibition of biofilm removes the biofilm-associated resistance mechanisms such as delayed antibiotic penetration, persister cells, and starvation-induced stress mediated growth repression. While modulation of gene expression and quorum sensing attenuate bacterial virulence, curcumin associated membrane damage may facilitate antibiotic influx. Sasidharan *et al.* used checkerboard test was used to determine *in vitro* synergy of antibiotics and curcumin and found the significant synergy of curcumin-1 with third generation cephalosporins against MTCC strains of five bacterial agents of diarrhea.[6] Mun *et al.* found decreased MIC of oxacillin, ampicillin, ciprofloxacin, and norfloxacin against 10 MRSA in the presence of curcumin.[14] In another study, disc diffusion synergy test was carried out with subinhibitory concentration (25 μg/mL) of curcumin against 10 strains of *S. aureus* and amikacin, gentamicin, and ciprofloxacin was found to have good antibacterial synergy with curcumin.[7] In our study, we utilized the same principle for testing larger number of bacterial strains. Since none of our isolates had curcumin MIC < 64 μg/mL, we used 32 μg/mL as the subinhibitory concentration for testing antibacterial synergy. In our study, all Gram-positive isolates ($n$ = 15) showed resistance to penicillin, erythromycin, and ciprofloxacin and had no resistance to vancomycin. The presence of curcumin rendered 4, 2, and 8 *S. aureus* strains sensitive to penicillin, erythromycin, and ciprofloxacin. Similarly, an increase in the ZOI resulting in AST change was noted in Gram-negative organisms [Table 3]. The synergistic

effect in terms of improved susceptibility was highest with amikacin (18 isolates), followed by gentamicin (16 isolates), cefepime, ceftriaxone, and imipenem. Meropenem and ampicillin AST were least affected. Our results are in keeping with other studies, which also found the antibacterial synergy of curcumin with aminoglycosides, cephalosporins, and quinolones.[6,7,14] Although one study reported mixed response in synergy tests with a decrease in zone suggesting antagonism for nalidixic acid, no such antagonism was observed in our study.

## Conclusions

The results of this study suggest that curcumin may be effective with ciprofloxacin for Gram-positive biofilm forming bacterial infections and amikacin, gentamicin, and cefepime for Gram-negative biofilm forming bacterial infections as combination therapy. It may widen the choice of antibiotics in case of isolates with intermediate susceptibility or marginally low cutoff for sensitivity.

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**Conflicts of interest**
There are no conflicts of interest.

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