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A review of the antimicrobial activity of clavulanate

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Clavulanate is a broad-spectrum β-lactamase inhibitor, with activity against many of the chromosomally and plasmid-mediated β-lactamases of both Gram-positive and Gram-negative bacteria. Although clavulanate has minimal antibacterial activity *in vitro*, accumulating evidence suggests that it may have an effect on pathogenic bacteria regardless of β-lactamase production. Like other β-lactams, clavulanate has been shown to bind to penicillin-binding proteins (PBPs) in Gram-positive and Gram-negative bacteria. It was found to bind selectively to PBP3 in *Streptococcus pneumoniae*. It has been suggested that complementary binding to different PBPs and subsequent effects on autolysis contribute to the enhancement of the activity of other β-lactams by clavulanate. In addition, co-amoxiclav has been shown to enhance the intracellular killing functions of human polymorphonuclear cells (PMNs) in studies undertaken with β-lactamase-producing and non-β-lactamase-producing strains of bacteria. These data from *in vitro* and cell culture systems have been reflected *in vivo*, where clavulanate enhanced the activity of amoxicillin against non-β-lactamase-producing organisms. Further studies are required to determine whether the effects seen within *in vitro* and *in vivo* animal studies have clinical significance.

Keywords: β-lactamase, clavulanate, *Streptococcus pneumoniae*

Introduction

Clavulanate is a bicyclic β-lactam that does not possess either the penicillin or cephalosporin nucleus. It is a metabolite found in cultures of *Streptomyces clavuligerus* and was isolated during the early 1970s through a programme of natural product screening designed to discover potential inhibitors of β-lactamases.[1,2] Clavulanate was the first clinically useful β-lactamase inhibitor to be described in the literature,[2] and is an irreversible 'suicide' inhibitor of intracellular and extracellular β-lactamases, demonstrating concentration-dependent and competitive inhibition. It has a high affinity for the class A β-lactamases.[1,3,4] This wide range of β-lactamases, which includes the plasmid-mediated TEM and SHV enzymes, is found frequently in members of the Enterobacteriaceae, *Haemophilus influenzae* and *Neisseria gonorrhoeae*. The chromosomally mediated β-lactamases of *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Bacteroides fragilis* and *Moraxella catarrhalis* are also inhibited, as are the extended-spectrum β-lactamases.[2] The frequency of β-lactamase-mediated resistance has continued to rise over the years, but the majority of clinically significant β-lactamases are inhibited by clavulanate.[5]

The pharmacokinetic characteristics of clavulanate supported the development of combined therapy regimens with amoxicillin and ticarcillin,[5] and the therapeutic success of these combination drugs is well recognized. Clavulanate formulations have been used widely and effectively in the treatment of a broad range of clinical infections for nearly 20 years.[3,5]

Clavulanate also displays limited antibacterial activity.[2,3] However, accumulating evidence suggests that clavulanate may influence the activity of β-lactam antimicrobials against pathogenic bacteria by mechanisms other than the inhibition of β-lactamase. This review explores the evidence for an interaction between clavulanate and other β-lactam antimicrobials against β-lactamase-negative bacterial strains. The activity of penicillins and other β-lactams in the presence or absence of clavulanate will be described relative to penicillin-binding protein (PBP) interaction and interaction with the host immune system. The relationship of these *in vitro* studies to observations in experimental infections will also be discussed.

Antibacterial spectrum

Clavulanate has a broad antibacterial spectrum, encompassing both Gram-negative and Gram-positive bacteria and anaerobes[1,2,6–16] (Table 1). Clavulanate is least active against *Pseudomonas* spp. and the enterococci (MICs 125–512 mg/L), followed by members of the Enterobacteriaceae and *H. influenzae* (MICs 12–125 mg/L). Greater activity is seen against *Bacteroides* spp. and other anaerobes, *M. catarrhalis*, staphylococci and streptococci (MICs 4–50 mg/L). Clavulanate is active against *Neisseria* spp. (MICs 1.25–4 mg/L) and

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Table 1. Antibacterial spectrum of clavulanate[1,2,6–16]

Organism[a]	MIC (mg/L)
Typicals	
Staphylococci	
Staphylococcus aureus and coagulase-negative strains	6.25–50
Streptococci	
Streptococcus pneumoniae and *Streptococcus pyogenes*	8–32[b]
Enterococci	
Enterococcus faecalis and *Enterococcus faecium*	128–512
Haemophilus influenzae	25–125
Moraxella catarrhalis	4–32
Neisseria spp.	
Neisseria gonorrhoeae and *Neisseria meningitidis*	1.25–4
Bacteroides spp.	8–50
Peptostreptococcus anaerobius	12
Clostridium perfringens	25
Pseudomonas aeruginosa	125–512
Enterobacteriaceae	
Escherichia coli, *Klebsiella pneumoniae* and *Proteus* spp., including indole-positive strains, *Serratia* and *Enterobacter*	12–125
Atypicals	
Chlamydia spp.	
Chlamydia trachomatis	≤0.25–16[c]
Chlamydia pneumoniae	–
Legionella pneumophila	0.1–0.4, 2[d]

[a]β-Lactamase-positive and -negative strains.
[b]Excludes results for penicillin-resistant pneumococci.
[c]Median 1 mg/L; refers to concentration required to reduce number of inclusions in tissue culture by ≥99%.[8]
[d]Tissue culture.[13]

has good activity against atypical bacteria such as *Chlamydia* and *Legionella* (Table 1).

Effect of clavulanate on the *in vitro* activity of other β-lactams

Clavulanate has been investigated in combination with other antibacterials, including ticarcillin[8,17] and penicillin.[18] As co-amoxiclav has been the most therapeutically useful combination, most studies of clavulanate in combination with antibacterials have been with amoxicillin. This paper, therefore, primarily considers studies of clavulanate in combination with amoxicillin.

Streptococcus pneumoniae

The increase in *S. pneumoniae* resistant to current antimicrobials has caused concern over the last several years.[19] A number of studies have reported that, although MICs of co-amoxiclav are essentially similar to those of amoxicillin alone, there is sometimes a trend towards lower MICs for the combination.[14,20,21] These differences are minor, but *in vitro* MIC tests may not be sufficiently sensitive to demonstrate any modifying effect of clavulanate on the activity of amoxicillin against *S. pneumoniae*.

However, evidence from two *in vitro* studies does suggest that clavulanate may influence β-lactam activity against *S. pneumoniae* through complementary binding to PBPs.[22,23] Severin *et al.*[22] showed that clavulanate at one-third of the MIC reduced the penicillin MICs for two *S. pneumoniae* strains from 0.01 and 0.25 mg/L to 0.0025 and 0.06 mg/L, respectively. The clavulanate MICs for these two *S. pneumoniae* strains were 8 and 32 mg/L, respectively. The antibacterial synergy was thought to be a result of complementary binding of clavulanate and penicillin to PBP3 and PBP2.[22] Similarly, Cuffini *et al.*[23] observed morphological changes in a penicillin-resistant strain of *S. pneumoniae* after incubation in broth containing sub-MIC levels of amoxicillin with clavulanate. The cells became irregular, with an aberrant shape and volumes greater than those observed in the absence of antimicrobial. In contrast, in the presence of amoxicillin or clavulanate alone, cells were unchanged and similar to those in the control broth. The authors concluded that complementary binding to PBPs was a contributory factor to the increased activity of co-amoxiclav over amoxicillin alone against *S. pneumoniae*.

Atypical bacteria

Legionella pneumophila produces β-lactamases of low potency and both amoxicillin and ticarcillin have high *in vitro* activity against this organism in the absence of clavulanate.[6] Clavulanate alone has potent activity against *L. pneumophila* (Table 1), and the synergy that has been observed *in vitro* between clavulanate and amoxicillin or ticarcillin was therefore not thought to be due entirely to β-lactamase inhibition but also due to complementary binding of clavulanate and the two penicillins to PBPs.[6,11,22,24] However, against intracellular *L. pneumophila* (human fetal lung fibroblasts; MRC-5 monolayers), clavulanate was considerably more effective than amoxicillin or ticarcillin, suggesting that clavulanate penetrates cells more readily than the penicillins.[7]

Chlamydia trachomatis is most frequently isolated from polymicrobial infections of the genital tract. Beale *et al.*[25] tested the activity of clavulanate and amoxicillin ± clavulanate over a range of concentrations in tissue culture (McCoy cells). Antimicrobials were added 24 h after infection and the number of inclusions observed at 72 h of incubation (untreated cells = 100% inclusions). Co-amoxiclav and the separate components reduced the number of inclusions by 70–80%, with no significant differences between the antimicrobials.[25] In a similar study, the MICs of amoxicillin, ticarcillin, clavulanate and the respective clavulanate combinations were determined against 21 clinical isolates of *C. trachomatis*.[8] The antimicrobials were added to the tissue cultures 1 h after infection. The median MICs (the lowest concentrations to prevent the development of inclusion bodies) were in excess of the maximum concentrations used (ticarcillin > 960 mg/L; amoxicillin > 64 mg/L; and clavulanate > 32 mg/L). However, compared with simultaneously run controls without antimicrobial, each agent caused a ≥99% decrease in the number of inclusions at readily attainable concentrations in human serum. Although no synergy was observed, clavulanate and amoxicillin were similar in activity and were marginally more effective than ticarcillin.[8] These studies suggest, therefore, that at least in the two atypicals for which we have data, the good activity of clavulanate may contribute significantly to the potency of a combination.

Other common pathogens

Against penicillin-susceptible *Staphylococcus aureus*, Verbist[26] observed a trend for greater activity of amoxicillin plus clavulanate compared with amoxicillin alone. Of 44 clinical isolates tested, all

were inhibited by 0.5 mg/L of co-amoxiclav (2:1 ratio amoxicillin/clavulanate), whereas only 77% of these isolates were inhibited by this concentration of amoxicillin alone. The remaining strains were inhibited by the higher concentration of 1 mg/L.

Other *in vitro* studies[27] have indicated greater activity of co-amoxiclav compared with amoxicillin alone against β-lactamase-negative isolates. Periodontal β-lactamase-negative pathogens such as *Actinobacillus actinomycetemcomitans* and *Enterococcus faecalis* were more susceptible to co-amoxiclav than to amoxicillin alone.

Using a turbidimetric static system, clavulanate combined with cefalexin was shown to be more active than cefalexin alone against a β-lactamase-negative strain of *Escherichia coli*.[28] Bacteria exposed to the combination demonstrated reduced filamentation, with emergent spheroplasts, bulbous forms and empty sacculi of lysed bacteria.[29] This observation was considered indicative of synergy as a result of complementary binding to PBPs, with clavulanate preferentially binding to PBP2 giving rise to spherical forms, and the cephalosporins binding preferentially to PBP3 leading to filamentation.[28,29]

The *in vitro* experiments described above provide some evidence for a synergic effect of clavulanate on the activity of β-lactams mediated through PBP binding interactions, but also indicate that this effect varies depending on both the antimicrobial and the bacterial organism. A further factor that should be considered is the interaction with the host immune system.

Interaction with the immune system

Clinical efficacy is dependent upon a number of factors including not only intrinsic antibacterial properties but also a positive interaction with host defences. After exposure to antibacterials, the resulting alteration of cell-wall integrity and changes in bacterial expression of surface proteins, surface charges and hydrophobicity can influence rates of phagocytosis and the extent of intracellular killing of bacteria.[23,30] A number of studies with either β-lactamase-producing or non-β-lactamase-producing strains have investigated the effects of amoxicillin and clavulanate, separately or together, on the rates of phagocytosis and the intracellular killing functions of polymorphonuclear cells (PMNs).[23,30–33]

Effects on phagocytosis

Organ transplant patients require life-long immunosuppression that increases the risk of serious bacterial infections. These infections are likely to be related to impairment of the phagocytic response. In renal transplant recipients, phagocytosis and intracellular killing of *K. pneumoniae* was reduced in PMNs compared with healthy volunteers.[34] The addition of co-amoxiclav at 0.5 × MIC restored PMN activity to levels similar to those seen from healthy subjects.[34] Another study in patients on chronic haemodialysis reported similar results.[35] These results are supported by a further study against β-lactamase-producing strains of *K. pneumoniae* and *S. aureus* (amoxicillin MIC > 128 mg/L for both pathogens; co-amoxiclav MIC 16 mg/L for *K. pneumoniae* and 2.0 mg/L for *S. aureus*). Co-amoxiclav at 0.5 × MIC was shown to significantly enhance PMN uptake and intracellular killing of non-opsonized and pre-opsonized *K. pneumoniae* ($P < 0.01$, compared with control and amoxicillin alone).[30] Interestingly, although amoxicillin inhibited the uptake of non-opsonized *S. aureus*, the addition of clavulanate significantly increased both uptake and intracellular killing.[30] Similarly, for an opsonized and un-opsonized β-lactamase-negative, amoxicillin-susceptible strain of *S. aureus*,[33] overnight exposure to subinhibitory levels of amoxicillin and clavulanate (at 0.25 × MIC) enhanced phagocytosis.[33] Pre-exposure to clavulanate resulted in a further increase in phagocytosis. Also, increasing the relative amount of clavulanate increased phagocytosis. These effects were not observed with a penicillinase-producing strain.

A number of studies have been undertaken with strains of penicillin-susceptible and -resistant *S. pneumoniae*. Against a penicillin-resistant *S. pneumoniae* strain with an amoxicillin ± clavulanate MIC of 4 mg/L and a clavulanate MIC of 64 mg/L,[23] subinhibitory levels (0.5 × MIC) of amoxicillin alone inhibited phagocytosis in a similar manner to that observed for *S. aureus*,[30] but the addition of clavulanate significantly enhanced bacterial uptake by PMNs compared with the non-treated control ($P < 0.01$). Serum pre-opsonization did not influence the results. In studies where antimicrobials were added after ingestion had occurred, both amoxicillin and clavulanate significantly enhanced phagocyte intracellular killing, although co-amoxiclav was more effective, killing 92%, 92% and 90% of cells at 0.5, 1.0 and 1.5 h of incubation, respectively.[23]

In a study reported by Martin *et al.*,[32] killing curves were generated against a serotype-3 penicillin-susceptible *S. pneumoniae* strain (amoxicillin and co-amoxiclav MIC/MBC 0.01/0.01 mg/L) and a penicillin-resistant, serotype-9 strain (amoxicillin and co-amoxiclav MIC/MBC 1/2 mg/L) for a range of concentrations (amoxicillin 0.5–12.0 mg/L and clavulanate 0.1–2.5 mg/L). These concentrations mimic those found in human serum at therapeutic doses and covered the MIC/MBC values for the penicillin-resistant strain and were in excess of MICs for the susceptible strain.

For the penicillin-susceptible strain, there was no obvious enhancement of phagocytosis and intracellular killing when clavulanate was added to amoxicillin, though this would be expected as the antimicrobial concentration ranges were well in excess of the MIC and amoxicillin concentrations were rapidly bactericidal. However, against the penicillin-resistant strain, the addition of clavulanate to subinhibitory concentrations of amoxicillin caused a greater reduction in the growth rate than for amoxicillin alone. The extent of this reduction was even greater in the presence of PMNs. This order of results was also seen for the supra-inhibitory concentrations (≥4.0 mg/L), and at 3 h there was a significant reduction in cell numbers (>99.9%) in the presence of both co-amoxiclav and PMNs. Clavulanate alone was not tested but, in a previous study with a penicillin-resistant strain, Gomez-Lus *et al.*[31] demonstrated that subinhibitory levels of clavulanate, under similar test conditions, delayed growth rate and there was a transient reduction (≥50%) in the initial colony count.

The clear interaction between amoxicillin, clavulanate and the immune system against the penicillin-resistant strain described above was considered by the investigators as evidence for the use of co-amoxiclav for the treatment of more serious infections such as pneumococcal pneumonia, where enhancement of the immune response could be of particular importance.[32]

Interaction with host defence cells

Subinhibitory concentrations of co-amoxiclav have recently been shown to significantly increase the mRNA of the pro-inflammatory cytokines IL-8 and IL-1β in lipopolysaccharide (LPS)-stimulated PMNs when *K. pneumoniae* was added to the *in vitro* culture.[36] In the absence of *K. pneumoniae*, co-amoxiclav still induced mRNA expression of IL-1, TNF-α and IL-8 in LPS-stimulated and -non-stimulated PMNs.

A further example of direct interaction between co-amoxiclav and the immune system was seen in the studies by Hofbauer *et al.*,[37] where co-amoxiclav significantly ($P < 0.05$) increased the migration of PMNs through monolayers of endothelial cells. This difference was greatest when both types of cell were pre-treated with an antimicrobial; the response to pre-treatment was more marked in the endothelial cells. Amoxicillin alone has also been shown to increase adhesion and chemotaxis of PMNs.[37]

Further studies would be necessary to confirm and extend the findings of Reato *et al.*[36] and Hofbauer *et al.*[37] to determine the contribution of clavulanate to the observations. Overall, however, the interaction, whether direct or indirect, between the immune system and amoxicillin and clavulanate, either separately or together, was seen as being of possible therapeutic importance in the treatment of clinical infections.

In vivo animal studies

In vivo animal studies using experimental infection models are a very useful means to explore the therapeutic potential either of new agents or for extending clinical indications of the more established antimicrobials. The therapeutic outcome of experimental infection studies, as with clinical outcome, is based upon a number of factors. These include the mode of action of the antimicrobial under test, susceptibility of the invading pathogens and the host response. Pre-clinical studies with clavulanate combined with β-lactams, primarily amoxicillin, in which β-lactamase inhibition was a factor for therapeutic outcome have therefore been reviewed to determine whether the antibacterial characteristics of clavulanate observed *in vitro* are of significance *in vivo*.

Atypical bacteria

The *in vitro* activity of clavulanate against *C. trachomatis* (Table 1) has been reflected in studies in mice with experimental pneumonitis caused by this organism.[25] The study treatments were dosed in the mice to approximate serum levels achieved in humans after therapeutic dosing (250/125 mg amoxicillin/clavulanate). Over a period of 20 days, clavulanate protected 75% of the mice compared with no survivors in the control group. Amoxicillin alone was also effective (90% protection) and synergy was demonstrated with this particular mouse-virulent strain, as all the mice were protected when the agents were combined at a quarter of the dose level of each.

In an infection model of *L. pneumophila* pneumonia developed in leucopenic weanling rats,[11,12] clavulanate was highly effective in preventing development of the infection. The activity of co-amoxiclav was no greater than that of clavulanate alone and was similar to that of erythromycin, the standard agent. Amoxicillin alone was ineffective despite being active in MIC agar dilution tests,[11] presumably reflecting its lack of activity against intracellular *L. pneumophila*.[7] A similar order of results was obtained in studies with ticarcillin and clavulanate in the *L. pneumophila* pneumonia model in immunocompromised weanling rats in which ticarcillin alone was relatively ineffective.[17]

These studies suggest that the *in vitro* activity of clavulanate against atypicals is translated into a significant *in vivo* antibacterial effect. Co-amoxiclav was effective against murine pneumonia caused by either *C. trachomatis* or *L. pneumophila*,[11,12,25] and as effective as erythromycin and doxycycline against the latter, despite 10-fold differences between the MICs. Some synergy was observed for studies with the mouse pneumonitis strain of *C. trachomatis*,[25] but as yet no β-lactamase has been observed in the chlamydiae. The lack of *in vivo* activity of amoxicillin indicates that the efficacy in pre-clinical studies with *L. pneumophila* was dependent upon the activity of clavulanate and not β-lactamase production.[11]

Streptococcus pneumoniae

In a mouse lethal thigh infection model with two susceptible strains of *S. pneumoniae* (co-amoxiclav MIC 2 mg/L),[38] after 4 days of therapy, mortality was marginally higher in the amoxicillin group (20% and 40% for the two strains) than in the groups receiving amoxicillin/clavulanate, in which all mice survived. The doses in this study were chosen to give peak concentrations in serum of the same order as those reported in human serum after administration of a standard dosage (500/125 mg twice a day). Against a fully resistant strain (MIC > 4 mg/L), amoxicillin±clavulanate was ineffective. In contrast, all mice with infections caused by fully susceptible strains (≤1 mg/L) survived and no enhancement with clavulanate was observed, similar to results of other experimental infection studies with susceptible strains.[24,39]

The findings of *in vitro* studies by Cuffini *et al.*[23,30] and Severin *et al.*,[22] indicated that there may be a synergic effect between amoxicillin and clavulanate. To test whether this was demonstrated *in vivo*, an experimental respiratory infection was developed in weanling rats.[40] Animals were infected with one of three penicillin-resistant *S. pneumoniae* strains (amoxicillin and co-amoxiclav MICs 2 mg/L; clavulanate MIC 512 mg/L), or with a penicillin-susceptible strain (amoxicillin and co-amoxiclav MICs 0.01 mg/L; clavulanate MIC 16 mg/L). The rats received oral doses of amoxicillin with or without clavulanate. Doses were selected to give area under concentration–time curves in serum of the same order as those reported in human serum after administration of what was then the standard dosage; i.e. amoxicillin/clavulanate doses 250/125, 500/125 and 750/125 mg twice a day. Therapy commenced 24 h post-infection and continued for 2 or 3 days.

Bacterial numbers in the lungs for all three penicillin-resistant strains were significantly reduced by co-amoxiclav compared with amoxicillin alone ($P < 0.05$), and no deaths were observed in any of the amoxicillin- and co-amoxiclav-treated groups.[40] Clavulanate alone was included as a control in an infection study with one of the three resistant strains. It was found that whereas the untreated rats died from the infection, all rats treated with clavulanate (50 mg/kg) survived, although no reduction in the lung viable count was evident.

In order to determine whether it was possible to enhance the efficacy of amoxicillin against an infection caused by a penicillin-susceptible strain, sub-therapeutic dose levels of amoxicillin (10 mg/kg) were used; the efficacy of amoxicillin was significantly enhanced ($P < 0.05$) when co-administered with clavulanate (50 mg/kg). In previous studies with this strain, clavulanate alone at this dose level was not effective in reducing bacterial counts in the lungs.[40]

Although the studies of Smith *et al.*[40] clearly showed that the *in vivo* activity of amoxicillin was enhanced by clavulanate, the enhancement was dose-related relative to the susceptibility of the infecting organism; when doses of amoxicillin were too low to demonstrate any efficacy or not sufficiently high enough for the antimicrobial to be highly effective alone, the enhanced efficacy with clavulanate was either not significant or not apparent. To some extent, this observation agrees with the results obtained by Andes & Craig[38] where enhancement was seen only when the amoxicillin MIC was 2 mg/L and not at MICs above or below this level. As yet, no

in vivo studies have been undertaken to investigate the morphology of organisms exposed to amoxicillin and clavulanate to confirm whether complementary binding to PBPs contributes to the enhancement, as proposed by Severin *et al.* and other groups.[22,23]

Conclusions

It is well recognized that clavulanate is a potent β-lactamase inhibitor. However, this review has indicated that the intrinsic antibacterial properties of clavulanate could be of potential clinical significance as a means to enhance the activity of β-lactam antimicrobials against pathogenic bacteria regardless of whether or not β-lactamase is produced.

Complementary binding to PBPs could well be an explanation for the enhancement of antimicrobial activity observed with clavulanate. The results of the morphological studies of Cuffini *et al.*[23] with penicillin-resistant pneumococci strongly suggest an interaction at the cell-wall level and complementary binding of clavulanate and amoxicillin to PBPs. Synergy of this type is not unusual and has been observed for other antimicrobial combinations such as ampicillin/sulbactam,[41] cefpirome/sulbactam, cefpirome/clavulanate and amoxicillin/cefotaxime.[42] In addition, the studies reviewed here suggest that the addition of clavulanate to amoxicillin both increases bactericidal activity and enhances host defence by increasing the rate of uptake by PMNs and the rate of intracellular killing.[23,30–33] Co-amoxiclav has also been shown to have effects on PMN chemotaxis and adhesion.[36,37] These interactions could have an effect upon therapeutic outcome and may well have influenced the results of the *in vivo* studies with *S. pneumoniae*, particularly as PMNs are considered to be the first line of defence in experimental (and presumably clinical) pneumococcal infections.[43]

The enhancement of the activity of β-lactam compounds by clavulanate is intriguing, but the precise manner in which the antibacterial characteristics of clavulanate contribute to the successful therapy observed for co-amoxiclav formulations remains unclear. However, with the global increase in antibacterial resistance, the enhanced activity observed in the presence of clavulanate against non-β-lactamase-producing bacteria, particularly the pneumococci, may have clinical implications by contributing to the maintenance of the clinical efficacy of co-amoxiclav in respiratory infections.

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