Review Article

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Immunomodulation as an Additional Mechanism of Action of Amoxicillin – Clavulanic Acid/Clavulanate

Puneet Rijhwani

Author Affiliation:
Professor & HOD,
Department of Medicine,
Mahatma Gandhi Medical College
and Hospital, Riico Institutional Area
Sitapura, Tonk Road Jaipur
Rajasthan 302022 India.

Reprint Request:
Puneet Rijhwani
Professor & HOD,
Department of Medicine,
Mahatma Gandhi Medical College
and Hospital, Riico Institutional Area
Sitapura, Tonk Road Jaipur
Rajasthan 302022 India.
E-mail: puneet284@rediffmail.com

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Abstract

Immunomodulation is the process of modulating the induction, expression, amplification, or inhibition of the immune system in a positive or negative manner. Both immunosuppressants and immunostimulators act by either suppressing or stimulating the immune system against a pathogen or tumour. Amoxicillin and clavulanic acid (AMCA) is a well-known anti-microbial agent which has a broad spectrum of activity. Although it was originally developed to reduce bacterial resistance through its antimicrobial activity, this combination also possesses immunomodulatory action. The immunomodulatory effect of AMCA has further broadened the activity of amoxicillin against β-lactamase-producing strains such as *S. aureus*, *E coli*, *H. influenza,* and *K. pneumonia,* and the anaerobic *Bacteroides fragilis* (*B. fragilis*). This combination inhibits β-lactam inhibitor and protects amoxicillin from being degraded by a β-lactamase enzyme. The immunomodulatory effect of AMCA is elicited through various mechanisms such as interaction with the host system, phagocytosis, interaction with host defense cells, and transmigration of PMNs at the inflammatory site.

The present review article focusses on immunomodulatory effect of AMCA in bacterial infections. The review further discusses the effect of AMCA in respiratory tract infections (both upper and lower), otitis media, and Skin and Soft Tissue Infection (SSTI). In addition, the article discusses the mechanisms through which AMCA has shown established effect against various resistant bacteria which has made this combination the drug of choice for treating infections as listed above.

Keywords: Amoxicillin; Clavulanic Acid; Immuno-Modulation; Broad Spectrum; Infections.

Introduction

Immunomodulation is the process of modulating the induction, expression, amplification, or inhibition of the immune system in a positive or negative manner. Immunomodulators are the intrinsic or extrinsic substances that can modify or alter the immune system [1,2]. There are two types of immunomodulators: immunosuppressants and immunostimulators which either suppress or stimulate the immune system against a pathogen or tumour.[1]

Immune response of the human body is broadly divided into innate and adaptive. The innate immune response is an initial non-specific host defence mechanism against an injury or pathogen. On the other hand, adaptive or acquired immunity is a specific host defence mechanism against a pathogen [3]. Neutrophils or polymorphonuclear lymphocytes (PMNs) are essential innate immune cells which play a pivotal role in host defence against various bacterial and fungal infections [4]. During infection, host and/or pathogen-derived component recruits circulating neutrophils at the site of infection and activates host immune cells for microbicidal activity. PMNs bind and phagocyte pathogens that trigger the production of reactive oxygen species and the fusion of cytoplasmic granules with pathogen-containing

vacuoles. The combined effect of neutrophil reactive oxygen species and granule components result in killing of most bacteria and fungi [5].

There are several immunomodulators which fall under the class of natural or synthetic substances. The advantage of immunomodulators arises from their ability to activate innate and adaptive immune response, such as modulation of cytokines, which prepares the body to defend itself. For instance, immunomodulating agents such as azathioprine, 6-mercaptopurine, methotrexate, and mycophenolate mofetil suppress immune system thereby reducing inflammation in patients with inflammatory bowel disease, ulcerative colitis, and Crohn's disease [3].

On the other hand, Amoxicillin-Clavulanic Acid (AMCA), widely used as an oral broad spectrum antibacterial agent for over 35 years has the ability to modulate host immune system and inflammatory response both *in vivo* and *in vitro* in addition to its antimicrobial activity [6,7].

The present review article focusses on immunomodulatory effect of AMCA in bacterial infections with respect to phagocytosis and intracellular killing by enhancing host defense mechanism. In addition, the review discusses the effect of AMCA in respiratory tract infections (both upper and lower), otitis media, and Skin and Soft Tissue Infection (SSTI).

Spectrum of Activity

Clavulanic acid is an irreversible 'suicide' inhibitor of intracellular and extracellular β-lactamases which protects amoxicillin from being degraded by the β-lactamases. AMCA has broad spectrum of activity against amoxicillin-resistant bacteria such as Gram-negative and Gram-positive aerobic and anaerobic bacteria (Table 1) [8]. A study evaluating the effect of AMCA showed that it decreased 99% inclusions of *Chlamydia trachomatis (C. trachomatis)* in tissue culture of genital tract at minimum inhibitory concentration (MIC) (amoxicillin >64 mg/L; and Clavulanate >32 mg/L) as compared to ticarcillin (MICs >960 mg/L). Additionally, in penicillin-susceptible *Staphylococcus aureus (S. aureus)*, AMCA inhibited 44 strains at a concentration of 0.5 mg/L in comparison to amoxicillin alone (77%). The concentration of amoxicillin/clavulanate used was in a ratio of 2:1. Furthermore, AMCA is effective in periodontal β-lactamase-negative pathogens such as *Actinobacillus actinomycetemcomitans* and *Enterococcus faecalis* as compared to amoxicillin alone [9]. A study accessed MICs of amoxicillin and/or AMCA in comparison to penicillin in a collection of 5252 *S. pneumonia* and found that amoxicillin and AMCA MIC90s were one two-fold dilution lower as compared to penicillin MICs against all isolates of *S. pneumonia* [10].

Pharmacokinetic Properties

AMCA is administered both orally and parenterally. The pharmacokinetic properties of AMCA differs based on route of administration. On oral administration, AMCA is well absorbed from the gastrointestinal tract (GIT) and achieves peak plasma concentration in 40-120 min. For a single dose of 250/125 mg AMCA, the mean peak concentration is 4.2 mg/L for amoxicillin and 2.6 mg/L for clavulanic acid. The elimination ($T_{1/2}$) rate for amoxicillin (500 mg)/clavulanate (125 mg) are 63 and 60 min respectively as evaluated in healthy individuals. Amoxicillin is excreted in urine whereas clavulanate is excreted through faeces, urine, and lungs [11].

On intravenous 1.2 gm bolus injection of AMCA, mean serum concentration for amoxicillin was approximately 95 mg and 16 mg for clavulanic acid. The $T_{1/2}$ was 1.033±0.11 h for amoxicillin and 0.838±0.04 h for clavulanic acid as evaluated in healthy subjects. The total body clearance (TBC) after 1.2 gm bolus injection was 14.232±2.65 L/h for amoxicillin and 12.90±5.05 L/h for clavulanic acid. The renal clearance for bolus injection of 1.2 gm was 184.25±36.07 mL/min for amoxicillin and 105.17±22.84 mL/min [12].

Mechanism of Action of AMCA

The Gram-positive bacterial cell wall is made of peptidoglycan. Formation of peptidoglycan is catalyzed by an enzyme called as transpeptidases, a penicillin-binding protein. β-lactams interfere with the synthesis of peptidoglycan by permanently binding to transpeptidases thus preventing the crosslinking between linear peptidoglycan polymer chains and disruption of cell wall synthesis [13].

When AMCA is used in combination, clavulanic acid inhibits β-lactam inhibitor and protects amoxicillin from being degraded by a β-lactamase enzyme which broadens the activity of amoxicillin that includes several bacteria normally resistant to amoxicillin (Figure 1) [13].

Immunomodulation as a Part of Mechanism of Action

Interaction with Immune-Host System

Clinical efficacy of an antibacterial agent depends on its interaction with host immune system that alters several factors such as cell-wall integrity, bacterial

Table 1: Antibacterial spectrum of AMCA [8]

Class of organisms	β-lactam producing and non-producing	MIC (mg/L)
Gram-positive aerobes	*S. aureus*[§]	≤8 µg/mL
	S. pneumoniae	≤0.5 µg/mL
Gram-negative aerobes	*E. coli*	≤8 µg/mL
	H. influenza	
	M. catarrhalis	
	K. pneumoniae	
	Enterobacter species	
	N. gonorrhoeae	≤0.06 µg/mL
Anaerobic bacteria	*Bacteroides fragilis*	≤8 µg/mL

[§]Staphylococci which are resistant to methicillin/oxacillin must be considered resistant to amoxicillin/clavulanic acid [8].

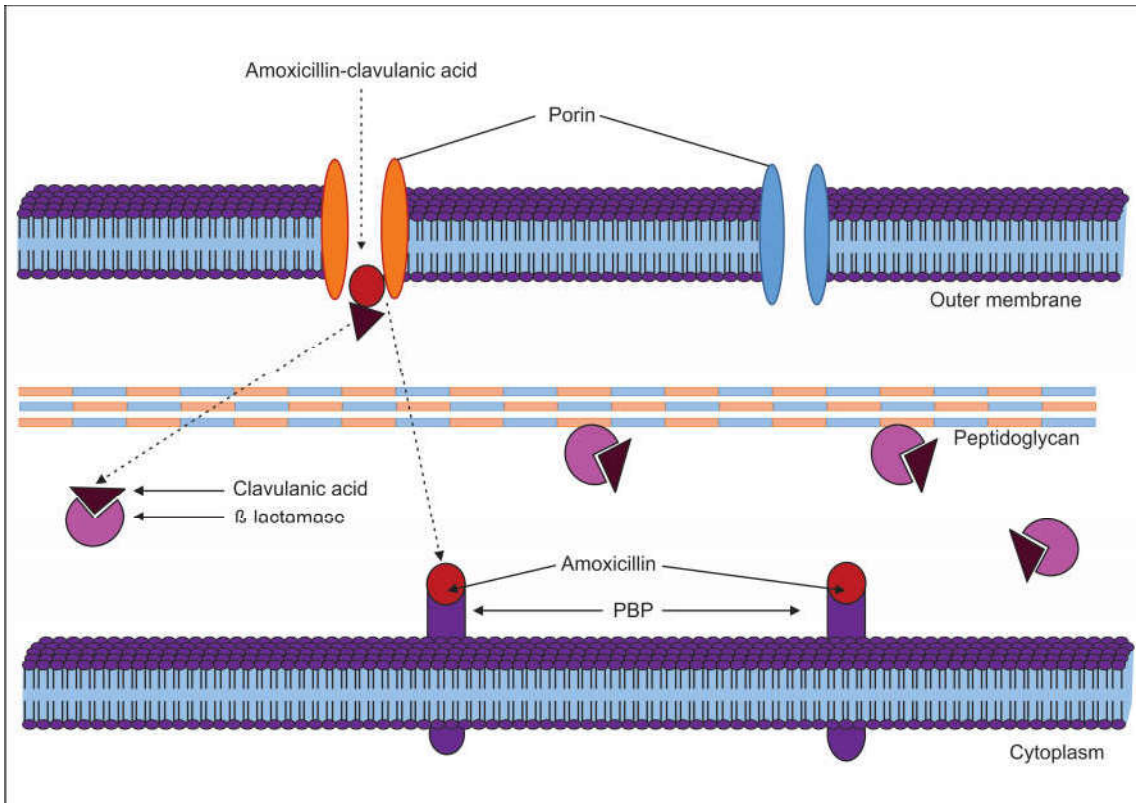

Fig. 1: Schematic presentation of AMCA binding sites inside a cell. Clavulanic acid binds and inhibits β-lactamase whereas amoxicillin bind to penicillin binding protein (PBP). Inhibition of β-lactamase protects amoxicillin from being degraded [12]. *(Dotted arrow: indicates the binding site for AMCA; solid arrow: indicates the names of the molecules.)*

expression of surface proteins, surface charges, and hydrophobicity that trigger phagocytosis, and intracellular killing of bacteria. Effect of AMCA has been studied in combination or alone on phagocytosis and intracellular killing by PMNs [9].

Effect on Phagocytosis

Clavulanic acid, a potent β-lactamase inhibitor, in combination with amoxicillin demonstrates synergistic effect against several infections. One of the mechanisms through which AMCA exhibits its action includes phagocytosis. Phagocytosis is basically a process through which cells are engulfed by larger molecules into vacuoles through a clathrin-independent process.

The process of phagocytosis plays a crucial role in the host defense mechanism. AMCA combination causes phagocytosis by stimulating PMNs which leads to secretion of neutrophil extracellular traps (NETs). The NETs are threads of extracellular DNA containing antimicrobial proteins which initiates phagocytosis thereby killing bacteria [14].

The phagocytic activity of AMCA has been evaluated in several *in vitro* studies from the past. Human PMNs induced phagocytosis is one of the most important host defence mechanisms against *S. aureus*. A study examined the role of AMCA alone or in combination on the interaction of human PMNs (*in vitro*) in strains of *S. aureus* (penicillinase-producing and non-penicillinase producing). At a MIC (¼), amoxicillin or clavulanic acid markedly increased the phagocytosis of both un-opsonized and opsonized non-penicillinase-producing *S. aureus* strains. Whereas, in penicillinase-producing *S. aureus* strains, clavulanic acid alone ($MIC_{1/4}$) markedly increased the uptake of un-opsonized bacteria. On the other hand, at different combinations of one-fourth the MIC of AMCA (4/1, 1/1, 1/8 and 1/32) there was increased uptake of human PMNs in non-penicillinase-producing *S. aureus* strain, however, there was no effect on penicillinase-producing *S. aureus* strain. AMCA (MIC 4/1) at higher concentration (100 mg/L), significantly ($p < 0.05$) increased intracellular killing of penicillinase-producing *S. aureus* strain. AMCA alone have no effect on intracellular killing of penicillinase-producing *S. aureus* strain [15].

Martin *et al*. reported the killing effects of amoxicillin induced by human PMNs or clavulanic acid against a serotype 3 penicillin-susceptible *S. pneumonia* strain [amoxicillin and AMCA (2:1) MIC/MBC 0.01/0.01 µg/ml] and a serotype 9 penicillin-resistant strain [amoxicillin and AMCA (2:1), MIC/MBC ½ µg/ml] at a range of concentrations (amoxicillin 0-12 µg/ml and clavulanic acid 0-2.5 µg/ml). The study demonstrated that addition of AMCA and human PMNs resulted in decrease of the penicillin-resistant initial inoculum (3×10^6 CFU/ml) at sub-inhibitory concentrations. At supra-MBC concentrations, clavulanic acid in combination with human PMNs significantly increased bactericidal activity of amoxicillin (≥ 92.4% and > 99.9% after 1 and 3 hours, respectively) [16].

The *in vivo* studies conducted have also demonstrated the phagocytic activity of AMCA. Organ transplant requires life-long immuno-suppression hence patients who have undergone organ transplant are immuno-compromised and susceptible to bacterial infections. Bacterial infections impair the phagocytic response in patients with an organ transplant and cause mortality or morbidity despite antimicrobial therapy [17]. A study accessing the effect of AMCA on the function of PMNs in patients who had undergone renal transplant reported that administration of AMCA significantly increased ($p<0.01$; more than 2-fold) the phagocytic activity of PMNs in ingestion of *K. pneumonia* in patients who had undergone renal transplant (N=29) as compared to drug-free control (N=48) [17]. The study further demonstrated a similar effect in healthy volunteers who took AMCA. The intracellular killing by PMNs was assessed by incubating the bacteria with the phagocytes. Addition of AMCA and PMNs from healthy volunteers significantly ($p<0.01$) enhanced bactericidal activity achieving mean killing values of about 98-99% of *K. pneumonia* [17]. Another study comparing the effect of AMCA on PMNs in chronic hemodialysis patients (N=32) as compared to healthy participants (N=48), reported that addition of AMCA (MIC 16 mg/L) significantly ($p<0.01$) restored the activity of PMNs in phagocytosis and killing of *K. pneumonia* as compared to control [18]. In addition, a comparative study between AMCA and ticarcillin/clavulanic acid (0.5 x MIC of the drugs) in patients with urinary tract infection (UTI) reported that the addition of clavulanic acid significantly ($p<0.01$) increased phagocytic and microbicidal activities at 30, 60 and 90 mins of both ticarcillin (83%, 93%, and 100%) and amoxicillin (95%, 99% and 100%) of human PMNs against *K. pneumonia* [19].

These studies demonstrate that clavulanic acid interacts with PMNs in potentiation of amoxicillin in phagocytosis of bacteria.

Interaction with Host Defense Cells

PMNs are the first line of defense mechanism involved in the inflammatory process against invading bacteria. PMNs can produce cytokines that may regulate the inflammatory pathways. The cytokines especially IL-8, TNF-α , and IL-1β generate appropriate signals in the development of defense against infectious agents or bacterial products or due to mechanical injury [2].

In a randomized clinical trial (RCT), the efficacy of oral enteric-coated AMCA (1 g + 250 mg, TID), methylprednisolone (40 mg/day, I.V.), and oral AMCA in patients with active ulcerative colitis (N=30) was determined. This study reported that the short-term use of enteric coated AMCA downregulated the intraluminal release of IL-8 and other inflammatory mediators [21]. A study showed that AMCA modulates PMNs *in vitro* to synthesize pro-inflammatory cytokines such as IL-8, IL-1, TNF-α, and IL-6 [20]. In an *in vivo* study, the effect of AMCA against 17 strains of *S. pneumonia* were accessed in a cyclophosphamide-induced neutropenic thigh infection model. At a minimum inhibitor concentration/s (MICs) of ≥ 2 mg/L of AMCA killed organism at 1.6 to 4.1 $\log_{10}$ colony forming unit (CFU)/thigh at 24 h [7].

These studies indicate the role of PMNs in the modulation of cytokines that may prove therapeutically effective against bacterial infections. However, more clinical data would be necessary to establish the extent of these findings.

Mechanism of Transmigration of PMNs at Inflammatory Site

Polymorphonuclear neutrophil leucocytes (PMNLs) migrates from vascular spaces to the inflammatory tissue site through endothelial cell monolayers (ECMs) to destroy invading microorganism. While passing through the ECMs, the PMNLs transforms into different morphological shapes from rounded to elongated, ruffled cells with pseudopodia to engulf microorganism. A study demonstrated the effect of co-amoxiclav on transmigration of leucocytes through endothelial cells and found that PMNLs and/or ECMs on pretreatment with co-amoxiclav significantly ($p<0.05$) potentiated the transmigration of leucocyte through ECMs [22].

Therapeutic Role of Amoxicillin and Clavulanic Acid

AMCA was originally developed to reduce bacterial resistance and extend the antibacterial activity of amoxicillin. Combination of the two antibacterial agents, amoxicillin and clavulanic acid has broadened the activity of amoxicillin against β-lactamase-producing strains such as *S. aureus*, *E coli*, *H. influenza,*and *K. pneumonia,* and the anaerobic *Bacteroides fragilis* (*B. fragilis*) [11]. Being the most widely used antibacterial agent, AMCA is effectively used for the treatment of several infections. Additionally, literature confirming the *in vitro* and *in vivo* activities of AMCA is vast. Several studies conducted in the past have shown its effect against a varying number of infections.

An *in vitro* study conducted to evaluate AMCA has demonstrated its efficacy against different types of bacteria causing dental infections in adult patients (N=47) with cure rates of 94% against *Microaerophilic Streptococci*, 67% against *Actinomyces israeli*, 100% against *S. aureus*, 60% against *B. fragilis,* 100% against *Peptostreptococcus*, 100% against *Enterococcus faecalis*, and 100% against *Anaerobic Lactobacilli* [23]. In another study, *in vitro* activities of ampicillin-sulbactam (A/S) and AMCA were assessed against *E. coli* strains (823) and *Klebsiella* spp (150) by standard agar dilution and disc diffusion technique. By agar dilution technique, in *E. coli* isolates, 51% were susceptible to AMCA and 47% to A/S, and in *Klebsiella* spp isolates, 38% were susceptible to AMCA and 34% to A/S. On the other hand, with disc diffusion technique, in *E. coli* isolates, 50% were susceptible to AMCA and 45% to A/S, and in *Klebsiella* spp isolates, 38% were susceptible to AMCA and 33% to A/S [24].

In an *in vivo* study, 500 mg oral dose of AMCA given three times a day was 100% effective in patients (N=32) with *Chlamydia trachomatis* endocervicitis [25]. Furthermore, a single oral dose of pharmacokinetically enhanced new oral formulation (2000/125 mg; 16:1 ratio) of AMCA was assessed in healthy male (N=24) and female (N=31) patients. The study recommended the use of this formulation for oral bacterial infection including beta-lactamase-producing organisms and strains with amoxicillin MICs ≤ 4 μg/ml [26].

AMCA is most commonly used in bacterial infections (upper or lower), acute otitis media (AOM), and soft tissue infections [11]. The subsequent sections discuss the effect of AMCA in respiratory tract infections (both upper and lower), otitis media, and Skin and Soft Tissue Infection (SSTI).

Upper Respiratory Tract Infection (URTI)

Virus that cause URTI include rhinovirus, coronavirus, influenza A and B, parainfluenza, respiratory syncytial virus (RSV), and adenovirus. These viruses cause inflammation that leads to the production of cytokines and other inflammatory mediators resulting in common cold. Viral URTI is further susceptible to bacterial invasion of *H.influenza*and *S. pneumonia*that colonize the nasopharynx [27].

A study evaluating pharmacokinetically enhanced formulation of AMCA extended release (XR) (2000 mg/125 mg; plasma concentration >4 μg/mL) was assessed against conventional formulations in patients with community-acquired respiratory tract pathogen (*S. pneumonia*). The study reported that the AMCA-XR would be extremely beneficial against *S. pneumonia* isolates having amoxicillin MICs (≥2 μg/mL but ≤ 4 μg/mL) in regions with a high incidence of resistant pathogens or in selected patients [28]. In addition, Anon *et al* in an open labelled non-comparative study evaluated the efficacy and safety of pharmaceutically enhanced AMCA (2000/125 mg) in patients with ABRS. Overall bacterial eradication was seen in 722/822 (87.8%) patients with one or more pathogen, in 246/264 (93.2%) patients with *S pneumonia*, in 29/30 (96.7%) of those with penicillin-resistant *S pneumonia,* and in 110/124 (88.7%) of patients with β-lactamase-

positive pathogens. In this study, the MIC of AMCA of 4/2 µg/mlor higher was sufficient to achieve bacterial eradication [29].

In a comparative *in vitro* study, a pharmacokinetically enhanced oral formulation of AMCA (2000/125 mg twice daily) was evaluated against 9172 bacterial respiratory isolates of *H.influenza, S. pneumonia,* and *M. catarrhalis as compared with* penicillin, amoxicillin/clavulanic acid (current formulation), amoxicillin, cefaclor, cefixime, cefprozil, ceftriaxone, cefuroxime axetil, azithromycin, clarithromycin, erythromycin, clindamycin, levofloxacin, doxycycline and trimethoprim/sulfamethoxazole. The study reported that at a current susceptible breakpoint of ≤2 µg/mL, 93.5% of *S. pneumonia* isolates were susceptible to AMCA whereas, at the PK/PD susceptible breakpoint of ≤ 4 µg/mL, 97.3% of *S. pneumonia* were susceptible to enhanced AMCA formulation. Out of 9172 respiratory isolates, 13% and 16.5% were penicillin intermediate and resistant respectively, whereas 25% were macrolide resistant and 21.8% trimethoprim/sulfamethoxazole resistant. In addition, against 21.9% of *H.influenza* β-lactamase producers, the observed resistance was 16.8% for trimethoprim/sulfamethoxazole however >99% of isolates were susceptible to AMCA, cefixime, ciprofloxacin, and levofloxacin at NCCLS breakpoints. Furthermore, macrolides were resistant to 13% of *Streptococcus pyogenes*. Agents such as AMCA, macrolides, cefixime, fluoroquinolones, and doxycycline were most active against *M. catarrhalis*. The study reported that enhanced formulation of AMCA and levofloxacin were the most active oral agents whereas ceftriaxone (I.V.) was highly effective against these pathogens. Overall, enhanced formulation of AMCA surpassed its activity because of its susceptibility to resistant strains in comparison to other antibiotics [30]. In another *in vitro* study conducted between 2000 and 2003 in the US for three consecutive respiratory tract infections, among 131 isolates of *S. pneumonia* responsible for acute bacterial rhinosinusitis (ABRS), 85% were susceptible to AMCA [27].

Lower Respiratory Tract Infection (LRTI)

LRTIs range from acute bronchitis and acute exacerbations of chronic bronchitis to pneumonia. The frequent etiological agents responsible for LTRI include *S. pneumonia*, *H. influenza*, Legionella spp, *Chlamydia pneumonia* (*C. pneumoniae*) *Mycoplasma pneumonia* (*M. pneumonia*) and influenza A virus [31].

In an *in vitro* study, Beale *et al*, compared and studied the activity of amoxicillin, AMCA, minocycline, and oxytetracycline. The study results showed a reduced incidence of inclusion formation in all three strains (*C. trachomatis* Sa2f, a clinical isolate *C. trachomatis* LB1 and *C. trachomatis* MoPn). Particularly good activity was observed in strain *C. trachomatis* Sa2f and a clinical isolate *C. trachomatis* LB1. In the same study, *in vivo* activity of amoxycillin (10 mg/kg), amoxycillin/clavulanic acid (10 + 5 mg/kg) and minocycline (5 mg/kg) protected MoPn (ATCC VR123) mice for over 21-days. However, the *in vitro* and *in vivo* data suggested that AMCA may have potential for the treatment of multi-microbial infections of *C. Trachomatis* [32].

In a randomized multi-centre clinical trial conducted from 2001-2002 in children (N=110), efficacy and safety of AMCA were evaluated against *H. influenza, S. pneumonia and M. catarrhalis*. The overall efficacy was 82.7% (95% CI 75.7%-89.8%) against *H. influenza, S. pneumonia and M. catarrhalis* with the bacterial eradication rate of 70.8% [33]. Another study showed that AMCA (500/125 mg; oral; TID) was effective in the treatment of LTRI caused by *H. influenza* and *Branhamella catarrhalis*. However, the relapse rate for *H. influenza* was 22%because the amoxicillin level was below (<0.05 to 0.54, µg/ml) the usual MICs [34]. Apart from this, AMCA (I.V. 1.2 g or oral 625 mg for 7-15 day) showed clinical improvement in 90.8% patients with pneumonia (N=16), bronchiectasis (N=5) and acute exacerbations of chronic bronchitis (AECB) (N=44) [35].

Acute Otitis Media (AOM)

AOM infection is a growing concern in children because of bacterial resistance caused by *S. pneumonia* and *H. influenza* [36].

In an investigator-blind comparative trial, high dose of AMCA (amoxicillin, 80 mg/kg/day; 10 days) or cefdinir (14 mg/kg/day; 5 days) was evaluated in children (N=330) with AOM. AMCA showed better cure rate (86.5%) as compared to cefdinir (71.0%) [37]. In addition, Hoberman *et al*, reported that in children (N=144) with AOM, AMCA showed reduced initial symptoms by 35%, 61%, and 80%, and placebo (N=147) by 28%, 54%, and 74% by day 2, 4 and 7, respectively, AMCA further alleviated sustained symptoms by 20%, 41% and 67% as compared to placebo 14%, 36% and 53%, respectively. Apart from this, the study reported, reduced overall symptom burden and the rate of persistent signs of acute infection with AMCA as compared to placebo [38].

A study evaluated a high dose of amoxicillin-clavulanate (90/6.4 mg/kg/d in 2 divided doses for

10 days; N=249) in comparison to azithromycin (10 mg/kg for 1 day followed by 5 mg/kg/d for 4 days; N=245) in children. The study reported significant (p<0.01) improvement with the use of amoxicillin-clavulanate (90.5%) as compared to azithromycin (80.9%). Amoxicillin-clavulanate demonstrated improvement in 96.0% of *S. pneumonia* infections and 89.7% of *H. influenza* infections as compared to azithromycin 80.4% for *S. pneumonia* and 49.1% for *H. influenza [39]*.

Skin and Soft Tissue Infection (SSTI)

SSTIs are commonly seen in children ranging from superficial impetigo to more serious subcutaneous cellulitis and skin abscesses [40].

In an RCT, levofloxacin (750 mg, intravenous/oral) was compared with a single ticarcillin-clavulanate (TC) (3.1 g intravenous/4-6 hours) or in combination with amoxicillin-clavulanate (AC) (875 mg/12 hours). Both regimes were clinically efficacious with levofloxacin showing 84% and TC/AC 80%. In addition, microbial eradication rates were similar for Gram-positive (*Staph, Strep, Enterococcus*), Gram-negatives (*Proteus, Enterobacter,E. coli, Pseudomonas*), and anaerobes i.e., 91% (levofloxacin) and 84% (TC/AC) [41].

In an open-label, randomized, comparative trial, effect of AMCA, amoxicillin, erythromycin, and co-trimoxazole were evaluated in 50 children in each group with mild to severe pyodermas. The drugs were equally effective in treatment of mild to moderate pyoderma. In severe pyoderma, AMCA showed 96% cure rate (24/25), whereas the cure rate in other groups were; 90% (18/20), 83.3% (20/24), and 52% (13/25) in amoxicillin, erythromycin and co-trimoxazole group, respectively [42]. In addition, in a prospective double-blind controlled study, efficacy of amoxicillin and AMCA were compared in patients with non-bullous impetigo (N=51). On day 2 and 5, AMCA recipients showed clinical improvement of 71% and 95% as compared to amoxicillin recipients with 44% and 68% [43]. Furthermore, in a recent study, AMCA was recommended as a good option in the treatment of SSTIs caused by *Alcaligenes faecalis* [44].

Clinical Use of AMCA

Comparison of Cephalosporin with AMCA

Cephalosporins were discovered in 1945 as an important class of antibiotics known as β-lactam antibiotics. The mechanism of action of cephalosporin drugs is similar to AMCA. Cephalosporins inhibit enzymes involved in the synthesis of bacterial cell wall [13,45].

Based on the mechanism of action, cephalosporins are divided into three generation agents. First generation agents are active against Gram-positive organisms (Cephalexin and Cefadroxil). Second generation agents are active against both Gram-positive and Gram-negative organisms (Cefaclor, Cefpodoxime, Cefprozil, and Cefuroxime). Third generation agents have enhanced activity against Gram-negative organisms and effective against several resistant bacteria (Cefdinir, Cefditoren, Cefixime, and Ceftibuten) AMCA have a similar spectrum of activity as compared to second and third generation cephalosporin [46]. A comparison of clinical studies of second and third generation cephalosporin with AMCA are described in Table 2 [47-56].

Table 2: Comparison of Cephalosporins and Macrolides with AMCA

Comparison	Study location and Year	Number of Participants, Gender, Age and Dosage	Study Design	Outcome Measure	Antimicrobial Agents (number with outcome/total)	Conclusions
Cephalosporins						
AMCA *vs* Cefaclor in urinary tract infections (UTIs)[47]	USA	N=107 females; 53 females received Amoxicillin (250 mg) + Clavulanic acid (125 mg) and 54 females received Cefaclor (250 mg)	Randomized, double-blind	Clinical cure at 1 and 4 weeks after completion of treatment	AMCA group: 96% and 78% Cefaclor group: 92% and 75%	Both drugs were equally efficacious in the treatment of UTIs
Cefpodoxime proxetil *vs* AMCA in acute LRTI[48]	International 1990 to 1991	N=348 children (3 months to 10 yrs);234 received Cefpodoxime proxetil (8 mg/kg/day BID) and114 received	Randomized, multicenter international, open labelled	Clinical cure at 10 to 20 days after completion of the treatment	Cefpodoxime proxetil group: 95.2%AMCA group: 96.7%	Both drugs were bacteriologically efficacious and well tolerated.

Cefprozil *vs* AMCA in severe sinusitis[49]		AMCA (amoxicillin 40 mg/kg/day TID) N=278 participants; 140 in Cefprozil group (500 mg) (59 males, 81 females) and 138 in the AMCA group (500 mg/125 mg) (69 males, 69 females)	Randomized, multicenter open labelled	Clinical response was satisfactory in both group after completion of 2 weeks of the treatment	Cefprozil group: 80.8% (63/78)AMCA group: 81% (64/79)	Both drugs were equally efficacious for the treatment of severe acute sinusitis
Cefdinir *vs*. AMCA in acute suppurative otitis media[51]	International	N=752 patients (6 months to 12 yrs);248 received 14 mg/kg/day of cefdinir, 253 received 14 mg/kg (BID) of cefdinir and 251 received AMCA 13.3 mg/kg	Randomized, Multicenter	Clinical efficacious (cure rate + improvement rate) was equivalent in all treatment groups	Cefdinir (14 mg/kg/day): 90.8% (177/195)Cefdinir (7 mg/kg/BID): 88.7% (180/203)AMCA 89.9% (177/197)	All three treatments were equally efficacious and well tolerated however fewer side effects were associated with cefdinir.
Cefditoren pivoxil *vs* Amoxicillin/clavulanate in community acquired pneumonia (CAP)[52]	USA, 1998-2001	N=802 patients (404 men, 398 women; mean age 50 years; age range, 12-93); 266 received 200 mg cefditoren, 269 received 400 mg cefditoren and 267 received amoxicillin/clavulanate (875/125mg), all treatments were BID.	Randomized, Multicenter,Investigator-blind	Clinical cure rates were comparable in all treatment groups at both the posttreatment and follow-up visits	Post-treatment Cefditoren 200 mg group: 88% (125/142) Cefditoren 400 mg group: 89.9% (143/159) andAmoxicillin/clavulanate: 90.3% (138/159) On follow-up86.5% (128/148), 86.8% (138/159), and 87.8% (129/147) respectively.	All three treatments were efficacious both clinical and microbiologically, and both cefditoren and amoxicillin/clavulanate were well tolerated in CAP patients.
			Macrolides			
Azithromycin *vs* amoxicillin-clavulanate for the treatment of uncomplicated acute otitis media (AOM)[55]	USA, 1998-1999	N=350 children (6 months-12 years of age); 173 patients received azithromycin (30 mg/kg/day) or amoxicillin clavulanate (45 mg/kg BID)	Randomized, double-blind,multicenter, double-placebo	Clinical success rates were comparable for both the treatments	On day 12-16, clinical success rate for azithromycin and amoxicillin-clavulanate were 87% and 88%, and on day 28-32 75% and 75% respectively	No significant difference was detected in the efficacy between both treatments however, single-dose azithromycin was well tolerated as compared to BID amoxicillin-clavulanate in the treatment of AOM.
Clarithromycin *vs* AMCA in the treatment of community	Switzerland 1993-1995	N=112; 56 patients received clarithromycin lactobionate (500 mg	Randomized, open labelled	Clinical efficacy was similar for both	Clinical cure for clarithromycin treated patients was 86% and 84% for	Both treatments showed rapid

acquired pneumonia[56]	IV, BID, followed by 500 mg orally BID) and other 56 patients received AMCA (1.2 g IV, QID, followed by 625 mg, TID)	treatments	AMCA treated patients	recovery however clarithromycin was associated with slightly higher rate of side effects (phlebitis) because of IV administration and should be used with caution in patients on digoxin therapy

Comparison of AMCA with Macrolide

Macrolides are the drugs that contain macrocyclic lactone ring of 12 or more elements. This class of drugs has antibiotic, antifungal, prokinetic, and immunosuppressant activities. Macrolide antibiotics such as erythromycin, clarithromycin, and azithromycin have a broad spectrum of activity and were widely used for respiratory tract infection (RTI) caused by Gram-positive and Gram-negative organisms [57,58].

Macrolides modulate different cellular pathways that include intracellular calcium, mitogen –activated protein kinases (MAPKs) or transcription factors. In addition, it inhibits adherence of organism to epithelial cells, biofilm synthesis, quorum sensing molecules, and prevents the production of virulence factors [57]. A comparison of clinical studies of macrolide with AMCA is described in Table 2.

Adverse Effects

ACMA is generally well tolerated however has been associated with gastrointestinal adverse reactions and hepatotoxicity. In addition, use of AMCA is associated with liver damage and with higher risk of Steven-Johnson syndrome, purpura and hepatitis [59].

Conclusion

In conclusion, AMCA is an effective agent which is effective as an anti-microbial agent and an immunomodulator. The immuno-modulatory effect of AMCA is elicited *via* various mechanisms such as effect on phagocytosis, interaction with host defense cells, and transmigration of PMNs at inflammatory site has broadened the use of AMCA is several other conditions. Phagocytosis could well be an explanation resulting from the inhibition of β-lactamase by clavulanic acid further extending the activity of amoxicillin on PMNs releasing NETs and killing pathogens. However, more research is warranted to broaden the involvement of AMCA in pathogen killing through NETs. Further, activation of PMNs by AMCA may trigger nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kβ) inflammatory pathway thereby releasing pro-inflammatory cytokines (IL-8, TNF-α, and IL-1β) against infection or bacterial products. Finally, PMNs migrating the inflammatory or pathogenic site to invade the bacteria or pathogen and enhance the rate of killing.

The studies reviewed here may implicate a cascade of molecular mechanisms associated with exertion of immunomodulatory effect in host immune system by AMCA. Although the resistance to antibacterial agents increasing globally, clavulanic acid is effective against many bacteria particularly against *K. pneumoniae* which makes it the drug of choice for the treatment of infections of the respiratory system. In addition to the effect of AMCA in respiratory tract infections (both upper and lower), otitis media, and Skin and Soft Tissue Infection (SSTI), it is being used for the treatment of many other conditions as well. It has shown comparable efficacy and tolerability with cephalosporins and macrolides in multiple indications (Table 2). However; further studies evaluating the immuno-modulatory activity of AMCA are required to adequately evaluate its clinical use in other conditions.

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Conflict of Interests

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