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Rationale and evidence for the adjunctive use of N-acetylcysteine in multidrug-resistant infections

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Abstract. – Bacterial multidrug resistance has been a serious issue for healthcare systems in recent decades, responsible for many infections and deaths. Due to the increasing incidence of antimicrobial resistance and scarce treatment options, research is focused on finding possible therapeutic adjuvants able to increase the efficacy of antibiotics. The aim of this article is a review of available evidence on the use of N-acetylcysteine (NAC).

MEDLINE/PubMed was searched for appropriate keywords. *In vitro* and *in vivo* preclinical studies, clinical studies, reviews, and meta-analyses were retrieved and selected based on relevance. A narrative review article was written, reporting published evidence and the expert opinion of the authors.

Among possible adjunctive treatments, NAC has attracted the interest of researchers as a candidate for re-purposing. It is a widely used drug with a good tolerability profile, mainly used as a mucolytic agent, with antioxidant, anti-inflammatory properties and antibacterial activity. NAC acts on different mechanisms and stages of infections, resulting in inhibition of biofilm formation, disruption of preformed biofilms, and reduction of bacterial viability. NAC may be administered as an aerosol in many types of infections, including cystic fibrosis, bronchiectasis and infective flare of chronic obstructive pulmonary disease (COPD), and by the intravenous route in severe systemic infections (including septic shock) such as those caused by carbapenemase (KPC)-producing *Klebsiella pneumoniae* (Kp) and Carbapenem-Resistant *Acinetobacter baumannii* (CR-Ab).

A rationale exists for using NAC as an adjunctive treatment in multidrug-resistant (MDR) infections, based on *in vitro*, *in vivo* and clinical evidence, and future research is needed to identify candidate patients and optimal schedules for specific clinical conditions.

Key Words:
N-acetylcysteine, Multidrug resistance, Biofilm, Oxidative stress.

Introduction

Bacterial antibiotic resistance has been a serious issue for healthcare systems in recent decades, and is responsible for many infections and deaths[1]. The global burden of antimicrobial resistance was estimated to account for 4.95 million deaths in 2019[2]. Infections caused by multidrug-resistant (MDR) bacteria are generally associated with a poor prognosis and more than 40% mortality, especially in the presence of septic shock[3-5]. Multi-drug resistance is linked to most infectious agents, but it has been observed that almost 70% of this disease burden is caused by MDR Gram-negative bacteria (MDR-GNB)[1]. Indeed, in 2017, the WHO[1] listed MDR-GNB resistance among critical priorities for research and drug development.

Biofilms are surface-attached groups of microbial cells encased in an extracellular matrix that are significantly less susceptible to antimicrobial agents and host immune response than non-adherent planktonic cells. The characteristic increased resistance to host defenses and decreased susceptibility to antimicrobial agents make persistent infections difficult or impossible for the immune system to clear and be eradicated with antibiotics[6]. Biofilm microbial communities are implicated in many chronic bacterial and fungal infections[6-8]. Specifically, biofilm formation may be involved in the persistence and the exacerbation of many respiratory conditions, including ventilator-associated pneumonia, cystic fibrosis, bronchiectasis,

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chronic obstructive pulmonary disease (COPD), and upper respiratory airway infections[6,9].

As a result, MDR infections are responsible for high mortality; therefore, several strategies have been introduced to face this challenge. Indeed, early active therapy was found[10] to reduce mortality in patients with *Legionella* spp. *pneumophila*, and administrating macrolides/levofloxacin therapy within 24 hours from hospital admission was protective from death. Early appropriate antibiotic therapy, beginning within 24 hours from the collection of blood cultures, reduced mortality in patients with bloodstream infections by *Klebsiella pneumoniae* (Kp) carbapenemase (KPC) producing *Klebsiella pneumoniae*[11]. Administering at least two *in vitro* active antibiotics has been suggested as a strategy. However, treatment with either two or more *in vitro* active antibiotics is difficult to achieve in clinical practice because limited options are available to treat MDR bacterial infections, especially for CR *Acinetobacter baumannii* (CR-Ab)[12,13]. At the same time, a high number of drugs would not be beneficial[14,15]. Due to the increasing incidence of antimicrobial resistance and scarce treatment options, research[12,13] is focused on finding possible therapeutic adjuvants able to increase the efficacy of antibiotics for MDR bacterial infections.

Among possible adjunctive treatments, N-acetylcysteine (NAC) has aroused the interest of researchers as a candidate for re-purposed use. It is a widely used drug with a good tolerability profile, mainly as a mucolytic agent, with antioxidant and anti-inflammatory properties and antibacterial activity[16]. Indeed, several *in vitro* studies[17-21] demonstrated a synergistic interaction of NAC with antibiotics against MDR-GNB. NAC acts on different mechanisms and stages of infections, resulting in the inhibition of biofilm formation, disruption of preformed biofilms either in an early or in a mature phase, and reduction of bacterial viability in biofilms[21]. NAC was demonstrated to exert antimicrobial and antibiofilm activity, potentially achievable by topical administration, against *Stenotrophomonas maltophilia* and *Acinetobacter baumannii*[19,20].

Finally, NAC exhibited *in vitro* bactericidal activity against several clinically relevant CR-Kp and CR-Ab strains, both alone and in combination with several antibiotics[18].

This review presents available evidence on the possible use of NAC as an adjunctive treatment of MDR infections, the pharmacological mechanisms involved, the *in vitro* antimicrobial activity, and the published clinical experiences.

Methods

A review of the literature has been carried out. MEDLINE/PubMed was searched for appropriate keywords: "infection," "N-acetylcysteine," "multidrug-resistance," "antibiotic resistance" and "biofilm". *In vitro* and *in vivo* preclinical studies, clinical studies, reviews, and meta-analyses were retrieved; articles in English or English abstracts were considered. All retrieved articles were read and examined by authors and were selected based on relevance. This selection was based on the authors' clinical and scientific expertise. A narrative review article was written, reporting published evidence and the expert opinion of the authors.

Pleiotropic Effects of N-acetylcysteine

NAC has been used in clinical practice since the 1960s as a drug with mucolytic and anti-oxidant activity for respiratory diseases and treating acetaminophen poisoning[22]. Several of its pharmacologic activities are involved in the antibacterial effect, the antibiotics potentiation, and the support in treating infectious diseases.

NAC is a sulfur-containing amino acid. Its thiol group is responsible for a lytic activity resulting in a fluidifying action on mucous secretions, beneficial for respiratory conditions, as well as antioxidant and anti-inflammatory effects[16,23].

NAC is a precursor of glutathione (GSH), the main intra-and extracellular antioxidant system, and many of its effects are mediated by GSH replenishment[24]. NAC is deacetylated within the cell to L-cysteine, which is the rate-limiting amino acid in GSH synthesis[25]. Through the generation of L-cysteine, NAC acts as a hydrogen sulfide donor, a readily diffusible vasodilator, and an anti-inflammatory molecule[26].

NAC is used as an antioxidant agent, preferred to direct administration of GSH, because of its pharmacologic profile, with excellent safety and better oral and topical bioavailability than GSH[24,27].

Indeed, the replenishment of GSH in the cells activates many mechanisms. Sulfhydryl groups of GSH react with electrophilic metabolites in the cells and block reactive DNA metabolites and intermediates[25]. In the extracellular environment, NAC breaks the disulfide bond of the cysteinylated form of albumin in plasma, thus regenerating the free form of Cys34 of human serum albumin, representing the major extracellular antioxidant molecule[28].

Furthermore, a relevant mechanism in the antioxidant activity of NAC is due to the scavenging

of ROS and particularly hypochlorous acid and •OH, through the sulfhydryl groups. NAC molecules can also scavenge some reactive nitrogen species (RNS), which are responsible for the oxidation of lipids, proteins, and DNA[23,25].

NAC activates several anti-inflammatory mechanisms either by inhibiting oxidative stress or acting on inflammation mediators. NAC inhibits the activation of NF-κB mediated by oxidative stress and the dependent pathways leading to the upregulation of proinflammatory cytokines[29]. It promotes the transcription of phase II enzyme genes, downregulating inflammation and enhancing the stimulation of Nrf2, a high-sensitivity transcription factor involved in the cellular antioxidant response[30,31].

NAC has also been shown[10] to elicit an anti-inflammatory activity through reduced neurokinin A and, secondarily, IL-6, thus modulating a vicious circle between oxidative stress and neurogenic inflammation. *Via* a GSH-mediated mechanism, NAC improves the structural conformational integrity of α1-antitrypsin and enhances α1-antitrypsin transcytosis, thus reducing its inactivation and improving its cellular uptake and functions, resulting in cell protection from inflammation[33]. Furthermore, NAC scavenges hypochlorous acid and protects α1-antitrypsin from inactivation by the myeloperoxidase system *in vitro*[34,35].

Regarding mechanisms identified as directly involved in the antibacterial effects of NAC, the RNA sequencing of NAC-exposed planktonic cultures of *P. aeruginosa* revealed that NAC at the concentration of 8 mg/ml induced the following effects: (i) a Zn^{2+} starvation response that is known[17] to induce attenuation of *P. aeruginosa* virulence, (ii) downregulation of genes of the denitrification apparatus, and (iii) downregulation of the flagellar biosynthesis pathway. In addition, NAC thiol group might alter the redox state of bacterial periplasma, generating misfolding of the proteins, including enzymes such as carbapenemases, which accumulate in the cytoplasm and undergo exocytosis[18].

Activity of NAC on MDR Bacteria

NAC does not interfere with the activity of the most commonly used antibiotics[36], while it is known to potentiate antibacterial compound activities.

Recent evidence demonstrated the activity of NAC on MDR bacteria. *In vitro* studies[21] have shown that NAC may interact with bacterial biofilm in several phases, such as adhesion to surfaces, matrix production, and dispersal of preformed biofilm. NAC, alone or with ciprofloxacin, was able to inhibit the growth of *P. aeruginosa* both in the planktonic form and as a biofilm. Indeed, while *P. aeruginosa* strains grew in the presence of ciprofloxacin with dexamethasone and ciprofloxacin alone, no growth was found in the sessile or planktonic state when NAC (≥5 mg/ml) was used either alone or in combination with ciprofloxacin (Table I)[37].

Pollini et al[19] demonstrated a synergistic activity of 8 mg/L colistin with 8 mg/ml NAC against colistin-resistant and colistin-susceptible CR-Ab strains grown both in planktonic phase and as biofilms, and the effects were greatest with colistin-resistant strains, as a marked reduction of viable biofilm cells was observed at sub-minimum inhibitory concentrations (sub-MICs).

Stenotrophomonas maltophilia is a global opportunistic pathogen responsible for a wide range of human infections with growing incidence, including respiratory tract infections. It has intrinsic MDR and a phenotype high propensity to form biofilms, and these characteristics make *S. maltophilia* infections recalcitrant to treatment. Checkerboard assays of 18 *S. maltophilia* clinical isolates (three isolates were from cystic fibrosis, and two were trimethoprim-sulfamethoxazole-resistant strains) showed a synergism of colistin/NAC combinations against the strains with colistin MIC >2 μg/mL (n=13), suggesting that NAC might antagonize the mechanisms involved in colistin resistance. Nonetheless, time-kill assays revealed that NAC might also potentiate colistin activity in the case of lower colistin MICs. A dose-dependent potentiation of colistin activity by NAC was also clearly observed against *S. maltophilia* biofilms also at sub-MIC concentrations[20].

De Angelis et al[18] tested the *in vitro* activity of NAC, alone or in combination with beta-lactams (meropenem for CR-Kp, meropenem, and ampicillin/sulbactam for CR-Ab, respectively), against 30 strains of planktonic CR-Kp and CR-Ab. They found that NAC MIC50/90 were 5/5 and 2.5/5 mg/ml for CR-Kp and CR-Ab, respectively, and NAC enhanced beta-lactam activity. The killing studies[18] also showed a rapid and concentration-dependent activity of NAC alone; the addition of NAC to meropenem or ampicillin/sulbactam at subinhibitory concentrations induced a fast and lasting bactericidal activity that persisted over time. The scanning electron microscope analyses showed[18] that bacterial cells had morphological alterations following

Table I. *In vitro* studies on the effects of NAC on bacteria.

First author, year	Bacteria	Drugs	Main result
Lea et al[37], 2014	*P. aeruginosa* (planktonic or biofilm)	NAC alone NAC + ciprofloxacin	Growth inhibition
Pollini et al[19], 2018	Colistin-susceptible and colistin-resistant *Acinetobacter baumannii*	NAC + colistin	A static effect with the colistin-susceptible strain at sub-MIC colistin concentrations; (Time–kill assays) potentiation of colistin activity by N-acetylcysteine against colistin-resistant strains; a remarkable antibiofilm synergistic activity
Ciacci et al[20], 2019 MIC	*S. maltophilia*	NAC + colistin	Synergism with colistin MIC > 2 μg/mL, and also on bifilm at sub-concentration
De Angelis et al[18], 2022	CR-Kp and CR-Ab (planktonic)	NAC NAC + meropenem NAC + meropenem, and ampicillin/sulbactam	Enhanced beta-lactam activity NAC alone had bactericidal activity
Manoharan et al[38], 2020	Methicillin-resistant *Staphylococcus aureus*	NAC NAC + antibiotics	Bacteriostatic effect Biofilm disruption
Valzano et al[17], 2022	*P. aeruginosa* (biofilm)	NAC + colistin	Antibiofilm activity

N-acetylcysteine (NAC), *Klebsiella pneumoniae* (Kp), CR = carbapenem resistant *Acinetobacter baumannii* (CR-Ab), sub-minimum inhibitory concentrations (sub-MICs).

incubation with NAC alone and in combination with meropenem.

NAC had bacteriostatic effects on bacterial growth of planktonic methicillin-resistant *Staphylococcus aureus* (MRSA) when tested alone. A combination of antibiotics with 30 mM NAC resulted in ≥90% disruption of biofilms across all MRSA and methicillin-sensitive *Staphylococcus aureus*-tested strains. Confocal laser scanning microscopy showed that NAC treatment disrupted biofilm architecture. Polysaccharide production in MRSA biofilms in the presence of NAC was also reduced, and the intrinsic acidity of NAC was identified as a pivotal mechanism for biofilm disruption and degradation of matrix components[38].

Recently, the activity of NAC alone and combined with colistin was demonstrated on *P. aeruginosa* biofilms. While 8 mg/ml NAC alone had a limited and strain-dependent antibiofilm activity, 8 mg/ml NAC plus 2-32 mg/L colistin exerted a relevant antibiofilm synergistic effect on all strains evaluated[17].

In Vivo Experimental Studies

Studies[39-41] on animal models showed that organ damage was improved and microvascular dysfunction reduced following NAC administration in endotoxin-induced shock (Table II). Pretreatment with NAC attenuated organ dysfunction and damage by reducing the production of free radicals, tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β following lipopolysaccharide (LPS)-induced endotoxemia; post-treatment with NAC suppressed the release of plasma TNF-α, IL-6, and IL-10 in endotoxin shock, and decreased the markers of organ injury[39]. Administration of NAC with resuscitation fluid in a rat model[40] of LPS-induced shock improved renal oxygenation and reduced microvascular dysfunction preventing acute kidney injury. Pre-treatment with NAC, before inducing sepsis in rats by cecal ligation and perforation, significantly decreased the pathologic damage of kidney tissue, the levels of serum creatinine, blood urea nitrogen, plasma neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, and the expression of TNF-α, IL-1β, IL-6, and IL-8. Furthermore, apoptosis markers and apoptotic cell numbers were reduced in kidney tissues[41]. Therefore, modulation of inflammation, oxidative stress, and antiapoptotic effects cooperate to limit sepsis-induced tissue damage.

Clinical Experiences

A beneficial effect of NAC during infectious diseases has been reported for many years by

studies[42,43] on patients with septic shock. However, a meta-analysis[44] including 41 randomized clinical studies, published before 2012, questioned the safety and utility of intravenous NAC as adjuvant therapy in patients with systemic inflammatory response syndrome and sepsis. It must be acknowledged that the conclusions of the analysis need to be reappraised, as dosages, time of administration and administration routes were inconsistent in these studies; subsequent evidence was favorable for the use of NAC, and no bacteriological data on MDR were available.

Clinical studies on the antibacterial use of NAC are very scarce. However, NAC is a well-investigated drug with a long-ascertained safety, and its repurposing may take advantage of experiences in other settings. Indeed, NAC has a well-established role as a treatment for liver failure induced by acetaminophen intoxication. The mechanism has been shown[24,45,46] to be its ability to replenish the hepatic pool of GSH. It is widely used for respiratory conditions. Intravenous NAC has been used[47] in critically ill patients with respiratory conditions characterized by excessive and/or thick mucus production without safety issues. In two randomized trials[48,49], acute exacerbation frequency in stable COPD patients was significantly reduced in the group receiving high-dose NAC treatment for 1 year by its antioxidant and anti-inflammatory effects. Viral and/or bacterial infection is the main component of COPD exacerbation morbidity, being present in 78% of exacerbations[50]. Factors, such as reduction of bacterial adherence to ciliated epithelial cells and inhibition of respiratory syncytial virus infection, might be used to explain the better-than-placebo treatment effects of NAC[49]. Inflammatory markers, such as CRP and IL-8, and clinical outcomes, such as cough frequency and intensity, the difficulty of expectoration and lung auscultation, were improved by the treatment with oral NAC in a dose-dependent manner in patients experiencing an acute exacerbation of COPD[51]. Also, oral NAC for ~12±24 weeks reduced the risk of exacerbations and improved symptoms in patients with chronic bronchitis[52]. NAC has been demonstrated[53,54] to have a preventive role for postoperative pulmonary complications as an expectorant: it could have beneficial effects by, among other mechanisms, inhibiting the adherence of bacteria to ciliated epithelial cells and interfering with biofilm formation and disrupting biofilms.

The role of NAC antioxidant and anti-inflammatory activities is being widely investigated[24] in viral infections. Since the mechanisms of NAC effects in viral infections include its antioxidant and anti-inflammatory activities, which may also be beneficial in bacterial MDR infections, some of these studies are mentioned here. During the recent pandemic, encouraging results[55-60] were obtained in COVID-19 patients both in the early and advanced stages of the disease, with inhibition of viral uptake both for Delta and Omicron variants, reduced duration of hospitalization, lower mortality, improved ventilatory function, and less progression to ventilatory failure.

Regarding clinical experiences with bacterial infections, in a recent phase II randomized clin-

Table II. *In vivo* studies of on animal models of bacterial infection.

First author, year	Model	Drugs	Main result
Hsu et al[39], 2006	LPS-induced endotoxemia	NAC pretreatment NAC post-treatment	Reduced free radicals, TNF-α, IL-10 Reduced TNF-α, IL-6, and IL-10
Ergin et al[40], 2016	LPS-induced shock	NAC in resuscitation fluid	Improved renal oxygenation and reduced microvascular dysfunction
Fan et al[41], 2020	Cecal ligation	NAC pretreatment	Decrease kidney damage Decreased serum creatinine, blood urea nitrogen, plasma neutrophil gelatinase-associated lipocalin, kidney injury molecule-1 Decreased expression of TNF-α, IL-1β, IL-6 and IL-8

N-acetylcysteine (NAC), Interleukin (IL), tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS).

Table III. Clinical studies on the use of NAC in bacterial diseases.

First author, year	Type of Study	Setting	Treatment	Main result
Tse et al[48], 2013	Randomized trial	COPD	NAC 600 mg BID Placebo For 1 year	Reduced exacerbation frequency
Zheng et al[49], 2014	Randomized trial	COPD	NAC 600 mg BID Placebo For 1 year	Reduced exacerbation frequency
Zuin et al[51], 2005	Randomized trial	COPD	NAC 1,200 mg NAC 600 mg Placebo For 10 days	Reduced cough, CRP, IL-8 Reduced cough, CRP, IL-8
Stey et al[52], 2000	Systematic review	Chronic bronchitis	NAC for ~12±24 weeks	Reduced exacerbation frequency
Safe et al[61], 2021	Randomized trial	HIV-associated tuberculosis	NAC as adjunctive to standard therapy	Reduced circulating oxidative stress markers
Aisa-Alvarez et al[62], 2020	Randomized trial	Septic shock	NAC Other antioxidant	Increased antioxidant capacity
Oliva et al[47], 2021	Retrospective case-control study	ICU admitted septic shock due to CR-Kp or CR-Ab	IV NAC + antibiotics	Mortality with NAC+ antibiotics = 33.3% Mortality with only antibiotics = 56.7% No receiving NAC was independent risk factor for mortality

N-acetylcysteine (NAC), chronic obstructive pulmonary disease (COPD), *Klebsiella pneumoniae* (Kp), CR = carbapenem resistant Acinetobacter baumannii (CR-Ab), intensive care unit (ICU), Interleukin (IL), CRP (C-reactive protein), IV = intravenous.

ical trial (RIPENACTB study), using NAC as an adjunctive therapy during the first 2 months of anti-tuberculosis treatment was safe. In specimens from this study, NAC dampened the oxidative stress in peripheral blood in hospitalized patients with HIV-associated tuberculosis[61]. This study was based on experimental evidence demonstrating that NAC could limit *Mycobacterium tuberculosis* infection and disease in animal models by suppressing the host oxidative response and through direct antimicrobial activity[61].

In a randomized trial[62], patients with septic shock and multiorgan failure received different antioxidant adjunctive treatments with standard therapy for 5 days. The total antioxidant capacity was increased in the 18 patients who received NAC compared with baseline ($p<0.05$).

Reduced 30-day mortality was found in patients admitted to Intensive Care Unit (ICU) with septic shock caused by CR-Kp or CR-Ab, receiving adjunctive intravenous NAC with antibiotics. This was a retrospective, observational case-control study[47] (1:2) conducted in two different ICUs. Patients receiving NAC plus antimicrobials were compared with patients receiving antibiotics alone. The overall mortality was 48.9%, but mortality in the antibiotic plus NAC group was 33.3% *vs.* 56.7% in the antibiotic-only group ($p=0.05$). Not receiving NAC ($p=0.002$) and infection with CR-Ab ($p=0.034$) were independent risk factors for mortality. In contrast, therapy with two *in vitro* active antibiotics ($p=0.014$) and time since initial definite therapy ($p=0.026$) were protective[47]. Clinical experiences are summarized in Table III.

Discussion

The availability of adjunctive treatments to improve antibiotic activities is a mandatory area of research as MDR is a growing concern. As NAC is a well-investigated drug with a good safety profile, exerting pleiotropic effects, it has attracted attention as a possibly repurposed drug for MDR infections. Indeed, NAC was found to have antibacterial activity and potentiate some antibiotics on certain bacterial pathogens, including MDR ones and those grown in biofilms. In addition, as an antioxidant and anti-inflammatory compound, NAC may be used as an adjuvant to antimicrobial therapy for the treatment of severe infections

caused by MDR organisms, such as CR-Kp and CR-Ab, and it may be a beneficial supportive treatment in infectious diseases to reduce organ damage and protect from septic shock. Since septic shock is characterized by excessive and imbalanced production of pro-inflammatory cytokines, reactive oxygen species, and marked alteration of circulation, compounds able to counteract these effects find a rationale in treating this condition[47]. It must be remembered that data from studies on some MDR bacteria strains, either in the planktonic or the biofilm form, cannot be directly assumed and extrapolated for different molecules or strains. This requires a large research effort to identify candidate patients for adjunctive treatment with NAC. Furthermore, published studies are heterogeneous in terms of dosage and time of NAC administration; as an example, studies included in the systematic review by Szakmany et al[44] on the use of intravenous NAC were conducted in different settings, with dose regimens ranging from 25 mg/kg to 150 mg/kg, duration of treatment varied from single bolus dose to infusions up to seven days; this renders generalization of results highlights the need for further prospective studies.

Of note, NAC administration may be considered in many types of infections, including cystic fibrosis and bronchiectasis, as well as COPD (in the infective flare) using aerosol administration, and severe systemic infections (including septic shock) caused by KPC-producing Kp and CR-Ab with the early administration of intravenous NAC. Given the pleiotropic activities of NAC, we may speculate that NAC could also be active towards other multi-drug resistant organisms, including *Enterobacterales* producing carbapenemases other than KPC or non-fermenting organisms, such as *S. maltophilia* and *P. aeruginosa*.

With regard to the strains towards which NAC may possess a role, we may consider that at the moment, there are available molecules towards KPC-producing Kp, whereas for CR *A. baumannii* or other non-fermenting organisms (i.e., *S. malthophilia*), therapeutic options are still limited and the role of cefiderocol needs to be further investigated[63].

Indeed, we must acknowledge that multicenter observational prospective studies including patients with MDR infections and using homogeneous dosages, route (aerosol, intravenous, or both) and timing of NAC administration are necessary to better define the role of NAC in the clinical practice towards MDR infections.

Furthermore, *in vitro* investigations on NAC antibacterial activity against other carbapenemases, such as metallo-betalactamases (VIM, NDM), are warranted in order to extend its potential therapeutic role to different mechanisms of resistance.

Finally, mechanisms of action of NAC linking its antioxidant effects to the antibacterial and antibiofilm activity should be better understood, beyond the studies[17,18] demonstrating protein misfolding, and Zn^{2+} starvation response as pivotal events.

Conclusions

A rationale exists for using NAC as an adjunctive treatment in MDR infections, based on *in vitro*, *in vivo* and clinical evidence, with future research needed to identify optimal candidate patients and schedules for specific clinical conditions.

Conflict of Interest

AO has been an Advisory Board member for Zambon Italia. G.M.R and L.P have been Advisory Board members for Zambon Italia and have participated in scientific events financed by Zambon Italia. FT has no conflict of interest.

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Availability of Data And Materials

All data analyzed in this review are included in this article and/or its figures. Further inquiries can be directed to the corresponding author.

Informed Consent

Not applicable.

Ethics Approval

Not required.

Authors' Contributions

All Authors contributed to the definition and contextualization of the paper's contents, critically edited the manuscript, and approved its final version for submission.

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