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Beyond Antibiotics: MSC-Derived Secretome in Modulating Bacterial Viability, Biofilm Dynamics, and Resistance Gene Expression

Ajeng Destian Suparwi[1*], Endang Purwaningsih[2], Nunung Ainur Rahmah[2], Eko Setiawan[3]

*Coresponding author : ajengdestians@gmail.com

Affiliation:
[1] Doctoral Program (S3) Biomedical Sciences, YARSI University, Jakarta, Indonesia
[2] Department of Biomedical Sciences / Faculty of Medicine, YARSI University, Jakarta, Indonesia
[3] Department of Biomedical Sciences, Faculty of Medicine, Islamic Sultan Agung University, Semarang, Indonesia

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ABSTRACT

Introduction: Antimicrobial resistance (AMR), particularly in biofilm-associated and chronic infections, has exposed critical limitations of conventional antibiotics. Biofilms enhance bacterial persistence through structural protection, metabolic adaptation, and regulated resistance mechanisms, necessitating alternative therapeutic strategies. Mesenchymal stem cell (MSC)–derived secretomes have emerged as a promising cell-free antimicrobial platform capable of modulating both bacterial behavior and host responses.
Methods: A narrative review was conducted using PubMed, Google Scholar, and GARUDA databases to identify studies evaluating antimicrobial, antibiofilm, and resistance-modifying effects of MSC-derived secretomes. Eligible studies included in vitro, ex vivo, and preclinical models assessing bacterial viability, biofilm dynamics, and resistance-associated pathways. Due to methodological heterogeneity, findings were qualitatively synthesized.
Results: The literature search identified 4,949 potentially relevant articles from PubMed, Google Scholar, and GARUDA databases. After duplicate removal and eligibility screening, 13 studies met the inclusion criteria and were included in the qualitative synthesis. The selected studies demonstrated that MSC-derived secretomes suppress bacterial growth, disrupt biofilm formation and mature biofilm stability, restore antibiotic susceptibility in tolerant populations, and modulate resistance-associated gene expression. Key antimicrobial components included antimicrobial peptides, extracellular vesicles, cysteine proteases, and immunomodulatory mediators that exert both direct antibacterial and host-mediated regulatory effects.
Conclusion: MSC derived secretomes act as systems-level antimicrobial modulators rather than conventional bactericidal agents. By targeting bacterial viability, biofilm architecture, resistance pathways, and host immunity simultaneously, they represent a promising adjunctive strategy for managing biofilm-driven and drug-resistant infections in the post-antibiotic era.

Keywords: antimicrobial; biofilm; mesenchymal stem cell; secretome

INTRODUCTION

The rapid global rise of antimicrobial resistance (AMR) has severely limited the effectiveness of conventional antibiotics, particularly in chronic and biofilm associated infections[1]. Bacterial biofilms represent a dominant survival strategy in clinical settings, conferring profound tolerance to antibiotics and host immune defenses, while resistance is increasingly understood as a dynamic and adaptive

phenotype rather than a fixed genetic trait[2]. These limitations have prompted growing interest in alternative, non-antibiotic strategies capable of targeting bacterial fitness, community behavior, and host–pathogen interactions simultaneously.

Mesenchymal stromal cells (MSCs) have emerged as promising candidates in this context due to their potent paracrine activity. Rather than relying on direct cell engraftment, MSCs exert many of their biological effects through the secretion of a complex secretome composed of soluble proteins, antimicrobial peptides, extracellular vesicles, and enzymatically active factors. Early observations revealed that MSCs could reduce bacterial burden and improve survival in experimental infection models, even when administered without direct antimicrobial agents, suggesting a role beyond classical immunomodulation[3,4]. Subsequent studies demonstrated that MSC-derived secretomes possess intrinsic antimicrobial activity against a broad spectrum of clinically relevant pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus* (MRSA). These effects are mediated by secreted antimicrobial peptides such as β-defensin-2, hepcidin, and cathelicidin, as well as non-canonical antimicrobial factors, and can occur independently of direct cell–bacteria contact [5–7].

Importantly, the antimicrobial activity of the MSC secretome extends beyond inhibition of planktonic bacterial growth. Growing evidence indicates that MSC-derived secretomes actively interfere with biofilm formation, destabilize mature biofilms, and restore antibiotic susceptibility in biofilm-embedded bacteria an area where conventional antibiotics frequently fail [8–10]. These findings suggest that MSC secretomes target bacterial community organization and extracellular matrix integrity rather than relying solely on bactericidal mechanisms. In parallel, recent studies have begun to uncover the ability of MSC-derived secretomes to modulate antibiotic resistance phenotypes and resistance-associated gene expression. Functional attenuation of resistance has been observed through biofilm disruption, immune-mediated bacterial clearance, and, in selected models, direct regulation of bacterial resistance genes, indicating a capacity to bypass or suppress classical resistance mechanisms [7,11,12].

Together, these findings position the MSC-derived secretome as a multifaceted, biologically adaptive antimicrobial strategy that operates beyond the scope of traditional antibiotics. This review synthesizes current evidence on how MSC-derived secretomes modulate bacterial viability, biofilm dynamics, and antibiotic

METHOD

This study was conducted as a structured narrative review to synthesize current evidence regarding the antimicrobial, antibiofilm, and resistance-modifying effects of MSC derived secretomes. A comprehensive literature search was performed using PubMed, Google Scholar, and GARUDA (Garba Rujukan Digital) databases.

Search terms were applied individually and in combination using Boolean operators (AND, OR) and included “MSC secretome,” “MSC conditioned medium,” “extracellular vesicles,” “exosomes,” “antimicrobial activity,” “bacterial viability,” “biofilm,” “quorum sensing,” “antibiotic resistance,” and “resistance gene expression.” To ensure comprehensive coverage, the reference lists of selected primary studies and relevant review articles were manually screened to identify additional eligible publications.

Studies were included if they investigated MSC-derived secretome products, including conditioned media, extracellular vesicles, or soluble secreted factors, and reported outcomes related to bacterial viability, biofilm formation or disruption, antibiotic resistance, or resistance-associated gene expression. Eligible studies employed in vitro, ex vivo, or preclinical experimental models and were published in peer-reviewed journals in English. Studies focusing exclusively on whole-cell MSC therapies without analysis of secreted factors, studies involving non-bacterial pathogens, or reports lacking mechanistic insight into antimicrobial or resistance-modifying effects were excluded. Clinical

or veterinary studies were included only when antimicrobial outcomes were attributable to secretome-mediated mechanisms.

Relevant data were extracted from eligible studies, including MSC source, secretome preparation method, bacterial species investigated, experimental model, and reported antimicrobial or regulatory effects. Due to heterogeneity in experimental designs and outcome measures, a quantitative meta-analysis was not feasible; therefore, findings were synthesized using a qualitative narrative approach. The selected literature was organized thematically into antimicrobial-relevant secretome components, modulation of biofilm dynamics, and influence on antibiotic resistance mechanisms and resistance gene expression, enabling integrated analysis of direct antibacterial and host-mediated regulatory effects.

RESULT

Literature Search and Study Selection

The initial literature search across PubMed, Google Scholar, and GARUDA databases identified a total of 4,949 potentially relevant records. After removal of duplicate articles and preliminary screening based on titles and abstracts, studies that were not directly related to mesenchymal stem cell (MSC)-derived secretomes, antimicrobial activity, biofilm modulation, or antibiotic resistance mechanisms were excluded. Full-text assessment was subsequently performed on the remaining eligible articles.

Studies were excluded during full-text evaluation if they focused exclusively on whole-cell MSC therapies without characterization of secreted factors, investigated non-bacterial pathogens, lacked antimicrobial or resistance-related outcomes, or did not provide mechanistic relevance to secretome-mediated effects. Following the eligibility assessment, a final total of 13 studies met the inclusion criteria and were included in the qualitative narrative synthesis.

The included studies consisted primarily of in vitro, ex vivo, and preclinical experimental investigations evaluating the effects of MSC-derived conditioned media, extracellular vesicles, and soluble secreted factors on bacterial viability, biofilm dynamics, antibiotic susceptibility, and resistance-associated gene expression. The selected literature was subsequently organized into thematic categories comprising (1) antimicrobial-relevant secretome composition, (2) modulation of bacterial viability, (3) biofilm disruption and biofilm dynamics, and (4) modulation of antibiotic resistance phenotypes and resistance gene expression.

MSC-Derived Secretome: Composition and Functional Diversity

The antimicrobial activity of the MSC derived secretome arises from its complex and multifunctional composition, rather than from a single dominant effector. The secretome encompasses a broad range of bioactive components, including soluble proteins, antimicrobial peptides (AMPs), enzymatic factors, lipid mediators, cytokines, chemokines, and extracellular vesicles (EVs). Importantly, the qualitative and quantitative profile of these components is highly dependent on the MSC tissue source, activation state, and microenvironmental cues, underscoring the adaptive nature of the secretome[13,14].

Proteomic and functional analyses across multiple MSC sources have revealed the presence of antimicrobial-relevant molecules with both direct and indirect effects on bacterial survival. As summarized in Table 1, MSC secretomes derived from bone marrow, adipose tissue, umbilical cord, and peripheral blood contain AMPs such as LL-37, β-defensins, hepcidin, and cathelicidins, as well as enzymatic proteins including cysteine proteases and ATPases[5,6,15]. These components support membrane disruption, metabolic interference, and degradation of extracellular bacterial structures. Beyond direct antimicrobial activity, MSC secretomes are enriched in immunomodulatory and pro-

resolving factors that shape host responses to infection. Bone marrow–derived MSC secretomes have been shown to contain keratinocyte growth factor, IL-10, calreticulin, and specialized pro-resolving lipid mediators, which collectively promote immune homeostasis and enhance bacterial clearance in vivo[3,4,16]. These findings highlight that antimicrobial efficacy of the MSC secretome is frequently mediated through host-directed mechanisms, rather than exclusive bactericidal action. Extracellular vesicles represent a particularly important functional component of the secretome. EVs derived from MSCs carry membrane proteins, enzymes, and regulatory cargo capable of influencing both host immune cells and bacterial behavior. EV-associated markers such as CD9 and CD81, along with ECM-associated and IFN-γ–inducible proteins, have been identified in MSC secretomes and are thought to contribute to programmable antimicrobial and immunomodulatory responses[15,17]. The vesicular delivery of bioactive molecules may facilitate sustained and spatially targeted effects within infected tissues.

A defining characteristic of the MSC secretome is its dynamic and inducible nature. Exposure to bacterial products or inflammatory stimuli triggers enhanced secretion of AMPs and antimicrobial factors via pattern-recognition pathways, including TLR-dependent signaling[6].This adaptive responsiveness distinguishes MSC secretomes from conventional antimicrobial agents and suggests an evolutionarily conserved mechanism for sensing and responding to microbial threats.

Table 1. Antimicrobial-Relevant Components of MSC-Derived Secretome

MSC Source	Secretome Type	Key Components Identified	Functional Relevance	Evidence Type	Reference
Adipose-derived MSCs	Extracellular vesicles (EV) and membrane particles (MP)	ECM-associated proteins, membrane proteins, enzymatic proteins (ATPases, CD73), IFN-γ–inducible proteins	Supports capacity for extracellular matrix modulation, enzymatic activity, and programmable secretome profiles	Proteomic analysis (in vitro)	Tejeda-Mora et al., 2021
Bone marrow MSCs	Conditioned medium, EVs	Soluble proteins (e.g., calreticulin), extracellular vesicles	Immunomodulatory secretome capable of long-term hematopoietic reprogramming	Proteomics, EV characterization, in vivo	Ng et al., 2023
Bone marrow-derived MSCs	Conditioned medium, extracellular vesicles	Syndecan-2 (SDC2), EVs (CD9, CD81), specialized pro-resolving mediators (resolvin D1, lipoxin A4)	Supports immune modulation, resolution of inflammation, and host-mediated bacterial clearance	In vitro, in vivo (murine sepsis)	Han et al., 2021
Bone marrow–derived MSCs	Conditioned medium, in vivo secretome	LL-37, keratinocyte growth factor (KGF), IL-10	Antimicrobial peptide secretion and immune modulation enhancing bacterial clearance	In vitro, in vivo (rat pneumonia)	Devaney et al., 2015
Umbilical cord–derived CD362^{+} MSCs	Conditioned medium, in vivo secretome	Antimicrobial peptide hepcidin, cytokine-modulating soluble factors	Enhances macrophage phagocytosis, suppresses inflammation, supports host antimicrobial defense	In vitro + in vivo (CLP sepsis)	Gonzalez et al., 2020
Equine peripheral blood–derived MSCs	Conditioned medium	Cysteine proteases (cathepsins B, V), antimicrobial peptides	Direct antimicrobial activity; enzymatic degradation of biofilm matrix	In vitro (planktonic & biofilm)	Marx et al., 2020
Canine adipose-	Conditioned medium,	LL-37, β-defensin, hepcidin, lipocalin-2,	Direct antimicrobial activity, immune	In vitro + in vivo + clinical	Johnson et al., 2022

MSC Source	Secretome Type	Key Components Identified	Functional Relevance	Evidence Type	Reference
derived MSCs	activated secretome	surfactant protein D, IL-8	activation, synergistic antibiotic effects		
Equine peripheral blood-derived MSCs	Conditioned medium	CCL2, antimicrobial peptides (β-defensin, cathelicidin), cysteine proteases	Direct antibiofilm activity and indirect immune-mediated bacterial suppression	In vitro, ex vivo	Marx et al., 2021
Human adipose-derived MSCs	Lyophilized conditioned medium	Antimicrobial peptides (LL-37, β-defensin-2, hepcidin – literature-supported), EVs	Direct bactericidal activity against clinical Staphylococcus isolates	In vitro (clinical isolates)	Shaaban et al., 2025
Human umbilical cord blood–derived MSCs	Conditioned medium (bacteria-primed)	β-defensin-2 (BD2), TLR4-dependent secreted factors	Direct antimicrobial peptide secretion in response to bacterial stimuli	In vitro + in vivo	Sung et al., 2016
Equine peripheral blood-derived MSCs	Conditioned medium	Cystatin C, elafin, lipocalin-2, cathelicidin	Direct antibacterial activity via membrane depolarization and growth inhibition	In vitro (planktonic & biofilm)	Harman et al., 2017
Human umbilical cord MSCs	Conditioned medium (bacteria-stimulated)	LL-37, human β-defensin-2 (HBD-2)	Inhibits bacterial growth and contributes to antibiotic resistance modulation	In vitro (clinical isolates)	Ren et al., 2019

Modulation of Bacterial Viability by MSC-Derived Secretome

The ability of the MSC derived secretome to influence bacterial viability represents a foundational mechanism underlying its antimicrobial potential[18]. Unlike conventional antibiotics, which are typically optimized for rapid bactericidal activity against planktonic cells, MSC secretomes exert context-dependent bactericidal and bacteriostatic effects that vary according to bacterial species, secretome composition, and exposure conditions[19].

Multiple studies have demonstrated that MSC secretomes contain antimicrobial peptides (AMPs) capable of directly impairing bacterial growth. As outlined in Table 1, AMPs such as LL-37, β-defensin-2, hepcidin, cathelicidins, cystatin C, elafin, and lipocalin-2 are recurrently identified across MSC sources and species[5,6,15]. These peptides disrupt bacterial membranes, induce membrane depolarization, and interfere with essential metabolic processes, resulting in growth inhibition or bacterial death depending on concentration and exposure duration. However, an important and recurring observation is that MSC secretomes often suppress bacterial proliferation without inducing rapid or complete killing. In vitro studies using equine and human MSC conditioned media have shown sustained inhibition of *Escherichia coli*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus* (MRSA) growth, while avoiding the sharp bactericidal pressure characteristic of antibiotics[6,9]. This bacteriostatic-dominant profile may be advantageous in limiting selective pressure for resistance evolution, particularly in chronic infection settings.

Beyond AMPs, enzymatic components within the secretome further contribute to reduced bacterial fitness. Cysteine proteases secreted by MSCs degrade bacterial surface proteins and extracellular structures, indirectly compromising cell integrity and viability[9]. Additionally, EV-associated enzymes and membrane proteins identified in adipose- and bone marrow–derived MSC

secretomes may interfere with bacterial adhesion and nutrient acquisition, further restricting bacterial growth[15]. Importantly, direct antimicrobial effects of MSC secretomes are frequently complemented by host-mediated mechanisms that enhance bacterial clearance. In preclinical models of pneumonia and sepsis, administration of MSC secretomes resulted in significant reductions in bacterial burden in lung and systemic compartments, despite limited evidence of direct bactericidal activity in isolation[3,4,16]. These findings suggest that modulation of bacterial viability in vivo often arises from a coordinated interaction between secretome-derived factors and host immune responses.

Together, these studies indicate that MSC-derived secretomes modulate bacterial viability through multi-target, non-antibiotic mechanisms that include membrane disruption, enzymatic interference, metabolic suppression, and immune-assisted clearance. This functional profile provides a mechanistic basis for the downstream effects of MSC secretomes on structured bacterial communities, particularly biofilms, which are discussed in the following section.

Modulation of Biofilm Formation and Biofilm Dynamics by MSC-Derived Secretome

Biofilm formation represents a critical bacterial survival strategy that underlies persistent infection, immune evasion, and antibiotic tolerance. Within biofilms, bacteria exhibit altered metabolic states and coordinated gene expression patterns that collectively reduce susceptibility to antimicrobial agents[2,20]. Consequently, therapeutic approaches capable of disrupting biofilm development or destabilizing established biofilms are of particular importance in addressing chronic and device-associated infections [21].

Accumulating experimental evidence demonstrates that MSC derived secretomes interfere with multiple stages of the biofilm life cycle, including initial attachment, maturation, and maintenance. As summarized in Table 2, conditioned media and activated secretomes from MSCs significantly inhibit biofilm formation by clinically relevant pathogens such as *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Escherichia coli* in vitro[8,9]. These inhibitory effects are largely attributed to AMP-mediated membrane disruption and early interference with bacterial surface adhesion processes.

Beyond prevention of biofilm initiation, MSC secretomes exert pronounced effects on mature and antibiotic-tolerant biofilms. Studies using MRSA USA300 biofilms have shown that cysteine proteases present in MSC conditioned medium reduce total biofilm biomass and protein content, indicating degradation of extracellular polymeric substance (EPS) components essential for biofilm stability[9]. Similar antibiofilm effects have been observed in ex vivo skin explant models, where MSC secretomes significantly reduced viable bacterial biomass within established biofilms[8]. These findings demonstrate that MSC secretomes can penetrate and destabilize structured biofilm communities, rather than acting solely on planktonic bacteria. Importantly, disruption of biofilm architecture by MSC secretomes translates into restoration of antibiotic susceptibility. Combination treatment with MSC conditioned medium and conventional antibiotics has been shown to enhance antibiotic efficacy against MRSA biofilms, reversing biofilm-mediated tolerance both in vitro and ex vivo[8,9]. This synergistic effect suggests that secretome-mediated biofilm destabilization re-exposes bacteria to antibiotic action, overcoming one of the principal barriers to successful antimicrobial therapy. In addition to structural disruption, emerging evidence indicates that MSC secretomes influence biofilm-associated gene expression. In multidrug-resistant *Vibrio cholerae*, exposure to MSC conditioned medium resulted in marked downregulation of key biofilm matrix genes, including *bap1* and *rbmC*, which encode proteins critical for biofilm integrity[12]. This transcriptional suppression highlights a regulatory dimension of MSC secretome activity, extending beyond physical matrix degradation to reprogramming of biofilm-related genetic networks. The antibiofilm effects of MSC secretomes have also demonstrated relevance in vivo. In canine clinical cases of chronic, polymicrobial biofilm-associated infections, treatment with activated MSC secretomes often in combination with antibiotics resulted in clearance of persistent

infections that were refractory to standard antimicrobial therapy[10]. These translational findings support the clinical relevance of MSC secretome–mediated biofilm modulation.

Collectively, the evidence establishes MSC-derived secretomes as biofilm-disruptive and biofilm-reprogramming agents. By targeting both the physical matrix and the regulatory pathways governing biofilm development, MSC secretomes attenuate a major driver of antibiotic tolerance and chronic infection. This biofilm-centric activity provides a critical mechanistic link between reduced bacterial viability and the modulation of antibiotic resistance phenotypes, which is addressed in the following section.

Table 2. Modulation of Biofilm Formation and Dynamics by MSC Secretome

Biofilm Stage Affected	Bacterial Species	Secretome Component	Observed Effect	Mechanism	Model
Biofilm formation	MRSA, *S. aureus*	MSC CM	Inhibited biofilm formation	Protease-mediated matrix disruption	In vitro[9]
Mature biofilm	MRSA USA300	Cysteine proteases	Reduced biofilm mass and protein content	Protein degradation of EPS	In vitro[9]
Antibiotic-tolerant biofilm	MRSA	MSC CM + antibiotics	Restored antibiotic susceptibility	Biofilm destabilization	In vitro[9]
Chronic biofilm-associated infection	MRSP, *E. coli*, polymicrobial	Activated MSC secretome	Clearance of chronic infection	AMP secretion, immune activation, antibiotic synergy	In vivo (canine clinical) [10]
Mature biofilm	MRSA (*S. aureus*)	MSC-conditioned medium	Reduced live bacterial biomass	Protease-mediated matrix disruption	Ex vivo skin explant[Marx et al., 2021)
Antibiotic-tolerant biofilm	MRSA	MSC CM + antibiotics	Enhanced antibiotic efficacy	Biofilm destabilization	Ex vivo[8]
Biofilm formation	E. coli	MSC CM	Significant inhibition of biofilm growth	AMP-mediated membrane disruption	In vitro[6]
Biofilm formation	S. aureus	MSC CM	Reduced biofilm biomass	AMP-mediated effects	In vitro[6]
Biofilm matrix gene expression	*Vibrio cholerae* (MDR)	MSC conditioned medium ± chitosan NPs	Downregulation of *bap1* (96%) and *rbmC* (93%)	Transcriptional suppression of biofilm matrix proteins	RT-qPCR [12]

Modulation of Antibiotic Resistance, Biofilm Tolerance, and Resistance Gene Expression

While disruption of biofilm architecture significantly improves antimicrobial efficacy, accumulating evidence indicates that MSC derived secretomes also influence antibiotic resistance phenotypes through mechanisms that extend beyond physical biofilm destabilization. These effects include modulation of non-genetic antibiotic tolerance, regulation of resistance-associated gene expression, and enhancement of host-mediated bacterial clearance, collectively contributing to attenuation of antimicrobial resistance[9,22,23]. Experimental studies demonstrate that MSC secretomes reduce bacterial persistence in antibiotic-tolerant populations without exerting the high selective pressure typically associated with bactericidal agents. In planktonic and biofilm-associated models of *Staphylococcus aureus*, MRSA, and *Escherichia coli*, MSC conditioned media suppressed bacterial growth while maintaining susceptibility to conventional antibiotics, suggesting functional attenuation

of tolerance rather than selection for resistant[6,8,9]. This growth-modulatory profile may be particularly advantageous in limiting the emergence of resistance during prolonged treatment courses.

Table 3. Influence of MSC-Derived Secretome on Antibiotic Resistance, Biofilm Tolerance, and Bacterial Clearance

Resistance-Related Outcome	Bacterial Model	Secretome Factor	Observed Effect	Evidence Level	Interpretation
Host-mediated bacterial clearance	*Pseudomonas aeruginosa*, polymicrobial sepsis (*E. coli*, *Klebsiella*, *Enterococcus*)	MSC-CM, EVs, CpG-primed secretome	Reduced CFU in lung, blood, peritoneum; improved survival	In vivo (preclinical)	Immune reprogramming enhances bacterial clearance without direct killing[3,4,16,17]
Enhanced innate phagocytosis	*P. aeruginosa*, *E. coli*, mixed flora	MSC secretome (EVs, soluble factors)	Increased neutrophil and macrophage phagocytosis	In vitro + in vivo	Host-directed suppression of infection persistence [3,4,16,17]
Biofilm-mediated antibiotic tolerance	MRSA, *E. coli*, *S. aureus*	MSC CM, cysteine proteases, AMPs	Reduced viable biofilm bacteria; destabilized biofilm matrix	Direct experimental	Functional attenuation of non-genetic resistance [6,8,9]
Restoration of antibiotic efficacy in biofilms	MRSA biofilms	MSC CM ± antibiotics	Restored susceptibility to oxacillin, P/S	In vitro / ex vivo	Biofilm disruption reverses antibiotic failure[8,9]
Direct antimicrobial peptide activity	*E. coli*, MRSA, MRSE	LL-37, β-defensin-2, cystatin C, elafin, lipocalin-2	Growth inhibition, membrane disruption	Direct experimental	Non-antibiotic bactericidal and bacteriostatic effects[5,6]
Reduced selective pressure for resistance	Gram-positive and Gram-negative bacteria	MSC CM	Growth suppression without rapid killing	In vitro	Lower evolutionary pressure for resistance development[6]
Carbapenem resistance modulation	Imipenem-resistant *P. aeruginosa*	hUCMSC conditioned medium	Delayed resistance emergence; increased imipenem sensitivity	Direct experimental + ex vivo	Secretome attenuates resistance development[11]
Resistance gene expression regulation	*P. aeruginosa*	MSC-derived soluble factors	Restoration of *oprD2* mRNA expression	Bacterial RT-qPCR	Direct modulation of antibiotic resistance gene expression[11]
β-lactam and MLS resistance phenotype	Clinical MRSA / MRSE isolates	MSC secretome	High susceptibility despite *mecA*, *ermA/B* presence	Clinical in vitro	Secretome bypasses classical resistance mechanisms[7]
Adaptive antimicrobial activation	*E. coli*	TLR4-dependent MSC secretome	Inducible AMP secretion upon bacterial exposure	In vitro + in vivo	Dynamic, bacteria-responsive antimicrobial defense[5]

Beyond phenotypic tolerance, MSC secretomes have been shown to restore antibiotic efficacy in resistant bacterial populations. In MRSA biofilms, exposure to MSC conditioned medium in combination with β-lactam or protein synthesis inhibiting antibiotics restored susceptibility to agents

that were otherwise ineffective, highlighting a reversal of biofilm-mediated antibiotic failure [8,9]. Similar effects have been observed in multidrug-resistant clinical isolates, where MSC secretomes retained antimicrobial activity despite the presence of classical resistance determinants such as *mecA* and *erm* genes[7].

At the molecular level, emerging evidence indicates that MSC-derived secretomes can directly modulate antibiotic resistance gene expression. In *Pseudomonas aeruginosa*, exposure to human umbilical cord MSC–conditioned medium restored expression of the *oprD2* porin gene, which is critical for carbapenem uptake, thereby increasing sensitivity to imipenem and delaying the development of carbapenem resistance[11]. These findings provide direct evidence that MSC secretomes influence bacterial transcriptional programs associated with antibiotic resistance. In addition to direct gene regulation, MSC secretomes reduce resistance indirectly by enhancing host-mediated bacterial clearance. In models of pneumonia and polymicrobial sepsis, MSC secretome administration significantly reduced bacterial load in pulmonary and systemic compartments while improving survival outcomes [3,4,16,17]. These effects were associated with increased neutrophil and macrophage phagocytosis and improved immune coordination, reducing the duration of bacterial exposure to antibiotics and thereby limiting resistance selection pressure.

Importantly, MSC secretomes exhibit adaptive antimicrobial activation, with bacterial exposure triggering enhanced secretion of antimicrobial peptides through TLR-dependent pathways[5]. This inducible response allows the secretome to dynamically adjust its antimicrobial output in the presence of infection, further distinguishing it from static antibiotic therapies.

DISCUSION

Host–Pathogen–Secretome Interactions: A Triangular Model of Antimicrobial Modulation

The antimicrobial effects of MSC derived secretomes cannot be fully understood through a bacteria centric lens alone. Rather, accumulating evidence supports a triangular interaction model in which MSC secretomes simultaneously influence bacterial behavior, host immune responses, and the infection microenvironment. This integrated mode of action distinguishes MSC secretomes from conventional antibiotics and provides a conceptual framework for their ability to modulate bacterial viability, biofilm dynamics, and resistance phenotypes.

At the level of the pathogen, MSC secretomes exert direct effects on bacterial fitness through antimicrobial peptides, enzymatic factors, and vesicle-associated components that impair membrane integrity, suppress growth, and destabilize biofilms, However, these direct effects are frequently modest in isolation and become biologically meaningful when combined with host-mediated mechanisms[9,23]. This cooperative interaction reduces bacterial persistence without inducing the intense selective pressure associated with rapid bactericidal therapies[23]. From the host perspective, MSC secretomes act as potent immune modulators, enhancing innate immune functions while limiting excessive inflammation. In vivo studies demonstrate that MSC secretome administration increases neutrophil and macrophage phagocytosis, improves bacterial clearance from infected tissues, and promotes resolution of inflammation in models of pneumonia and sepsis[3,4,16,17]. These effects reduce bacterial burden indirectly, thereby decreasing reliance on high-dose or prolonged antibiotic treatment and limiting opportunities for resistance selection. Importantly, MSC secretomes also reshape the infection microenvironment, influencing factors such as extracellular matrix composition, inflammatory mediator levels, and cellular recruitment. Enzymatic degradation of biofilm matrix components and extracellular debris facilitates immune cell access to bacteria, while pro-resolving lipid mediators support tissue repair and restoration of homeostasis. This environmental remodeling further undermines bacterial survival strategies that depend on protected niches and chronic inflammation[24–26].

A defining feature of this triangular model is the dynamic and adaptive nature of MSC secretome activity. Exposure to bacterial products activates pattern-recognition pathways in MSCs, leading to inducible secretion of antimicrobial peptides and immune-regulatory factors[5]. This responsiveness enables MSC secretomes to adjust their antimicrobial output in real time, aligning host defense mechanisms with the evolving microbial threat. Such adaptability contrasts sharply with the static mechanisms of conventional antibiotics.

Through the combined modulation of pathogen behavior, host immunity, and the infection microenvironment, MSC-derived secretomes function as systems-level antimicrobial regulators. This integrated mode of action explains their capacity to suppress biofilm-associated tolerance, restore antibiotic susceptibility, and attenuate resistance gene expression without relying on single-target bactericidal activity. As such, MSC secretomes represent a biologically inspired strategy that aligns antimicrobial efficacy with immune homeostasis.

Therapeutic Implications and Translational Challenges

The multifaceted antimicrobial properties of MSC derived secretomes position them as promising candidates for adjunctive, non-antibiotic therapies in the management of drug-resistant and chronic infections. By simultaneously modulating bacterial viability, disrupting biofilms, restoring antibiotic susceptibility, and enhancing host immune clearance, MSC secretomes address several key limitations of conventional antimicrobial strategies[24–26].

From a therapeutic standpoint, MSC secretomes are particularly well suited for biofilm-associated infections, where antibiotic penetration and efficacy are severely compromised. Preclinical and translational evidence supports their potential application in chronic wound infections, implant- and device-associated biofilms, respiratory infections, and polymicrobial sepsis. Notably, clinical and veterinary case studies demonstrate that MSC secretomes, when used alongside antibiotics, can resolve infections that are otherwise refractory to standard antimicrobial therapy[10]. This supports the concept of MSC secretomes as antibiotic-sensitizing or resistance-modifying agents, rather than standalone replacements. An important advantage of MSC secretome based therapy is its cell-free nature, which mitigates several safety concerns associated with live cell administration, including engraftment variability, ectopic tissue formation, and tumorigenicity[27]. Secretome-based products may also offer greater flexibility in formulation, storage, and delivery, including topical, inhalational, or injectable routes, depending on the clinical indication[28]. Despite these advantages, significant translational challenges remain. One of the primary obstacles is batch-to-batch variability in secretome composition, driven by differences in MSC source, donor characteristics, culture conditions, and priming strategies. As highlighted throughout this review, antimicrobial efficacy is closely linked to secretome composition, underscoring the need for standardized manufacturing protocols and robust quality control metrics.

Stability and dosing represent additional hurdles. Many bioactive components of the MSC secretome, including antimicrobial peptides and EV-associated cargo, may be susceptible to degradation during storage or after administration. Optimizing formulation strategies, such as lyophilization or encapsulation, will be essential to preserve antimicrobial activity and ensure reproducible therapeutic outcomes[7]. Regulatory classification poses another challenge, as MSC-derived secretomes occupy an ambiguous space between biologics, advanced therapy medicinal products, and drug-like formulations. Clear regulatory frameworks will be required to define safety, potency, and release criteria, particularly as secretome-based products move toward clinical trials.

Finally, while current evidence supports a reduced risk of resistance development due to the multi-target and host-directed nature of MSC secretomes, long-term studies are needed to fully evaluate their impact on microbial evolution and resistance dynamics in clinical settings.

Future Directions

Future development of MSC derived secretome–based antimicrobial strategies will depend on improving mechanistic resolution, standardization, and translational scalability. While current studies demonstrate broad antimicrobial and antibiofilm activity, deeper characterization of active secretome componentsparticularly extracellular vesicle cargo, antimicrobial peptides, and enzymatic factors will be essential to define potency markers and optimize therapeutic formulations.

An important direction is the engineering and priming of MSC secretomes to enhance antimicrobial specificity and consistency. Inflammatory or pathogen-associated priming strategies may be leveraged to generate inducible, infection-responsive secretomes with enhanced activity against resistant pathogens, while minimizing off-target immunomodulatory effects. Integration of omics-based approaches will further enable rational design of optimized secretome profiles. Combination therapies also represent a key avenue for future research. The ability of MSC secretomes to destabilize biofilms and restore antibiotic susceptibility suggests strong potential for secretome antibiotic co-therapies, particularly in chronic and device-associated infections. Systematic evaluation of dosing, timing, and antibiotic pairing will be necessary to translate these synergistic effects into clinical practice.

Finally, well-designed preclinical and early-phase clinical studies are required to evaluate long-term safety, resistance dynamics, and therapeutic durability. Addressing these questions will determine whether MSC-derived secretomes can move from experimental antimicrobial adjuncts to clinically viable tools in the fight against antimicrobial resistance.

Limitations of the Review

This review has several limitations that should be acknowledged. First, the included studies demonstrated substantial heterogeneity in mesenchymal stem cell (MSC) sources, secretome preparation methods, bacterial species investigated, and experimental models, which limited direct comparison across studies. Second, most of the available evidence was derived from in vitro and preclinical investigations, while clinical evidence evaluating MSC-derived secretome–based antimicrobial therapies remains limited. Third, variations in secretome characterization, culture conditions, and priming strategies may influence the composition and biological activity of secretome products, potentially affecting reproducibility and translational applicability. In addition, because this study employed a structured narrative review approach rather than a systematic review or meta-analysis, the possibility of selection bias cannot be fully excluded. Finally, the absence of standardized protocols for secretome isolation, quantification, and potency assessment remains a significant challenge in interpreting and comparing antimicrobial outcomes across studies.

CONCLUSION

The escalating challenge of antimicrobial resistance demands therapeutic strategies that extend beyond conventional antibiotics and address the complex biological contexts in which bacterial persistence arises. The evidence reviewed here demonstrates that MSCderived secretomes represent a multifaceted, biologically inspired antimicrobial approach capable of modulating bacterial viability, disrupting biofilm dynamics, and attenuating antibiotic resistance phenotypes.

Rather than relying on single target bactericidal activity, MSC secretomes exert antimicrobial effects through a coordinated network of antimicrobial peptides, enzymatic factors, extracellular vesicles, and immunomodulatory mediators. These components act in concert to suppress bacterial growth, destabilize biofilm architecture, restore antibiotic susceptibility, and regulate resistance-associated gene expression, while simultaneously enhancing host-mediated bacterial clearance. This systems-level mode of action distinguishes MSC secretomes from traditional antimicrobial agents and may reduce selective pressure for resistance development.

Importantly, the antimicrobial efficacy of MSC secretomes emerges from their dynamic and adaptive nature, with secretome composition responding to inflammatory and microbial cues. This responsiveness enables context-dependent modulation of bacterial behavior and host immunity, aligning antimicrobial activity with tissue repair and immune homeostasis. Such properties position MSC-derived secretomes as promising adjunctive therapies for biofilm-associated, chronic, and drug-resistant infections.

While challenges related to standardization, stability, and regulatory classification remain, continued mechanistic refinement and translational development may enable MSC secretomes to complement existing antimicrobial strategies. By shifting the therapeutic focus from bacterial eradication to behavioral and environmental modulation, MSC-derived secretomes offer a compelling framework for addressing antimicrobial resistance in the post-antibiotic era.

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CONFLICT OF INTEREST

Author should state if there is any conflict of interest occurred.

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