Pathogen virulence genes: Advances, challenges and future directions in infectious disease research (Review)

YUN CHEN[1], XIAOLONG WU[1,2], CHENGCHENG XU[1], JIANXIANG HUANG[1,2], LINGYU ZHANG[1,3], PENG QIU[1], DANLING ZHENG[1], WANG CHEN[1] and SHUYAO ZHANG[1-3]

[1]Department of Pharmacy, Guangzhou Red Cross Hospital (Guangzhou Red Cross Hospital of Jinan University), Guangzhou, Guangdong 510220, P.R. China; [2]College of Pharmacy, Jinan University, Guangzhou, Guangdong 511436, P.R. China; [3]Department of Pharmacology, Shantou University Medical College, Shantou, Guangdong 515041, P.R. China

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Abstract. Pathogens, including bacteria, viruses and fungi, employ virulence genes to invade their hosts, circumvent immunity and induce diseases. The present review examines the categorization and regulatory mechanisms of virulence genes and their co-evolution with antimicrobial resistance. The present review focused on the fimbrial adhesion H adhesion gene of *Escherichia coli*, the spike protein gene of severe acute respiratory syndrome coronavirus 2 and the enhanced filamentous growth protein 1 (EFG1) morphological transition gene of *Candida albicans*, as well as their roles in host adhesion, immune evasion and tissue damage. Application of technologies, including multi-omics integration, artificial intelligence and CRISPR-based genome editing, is discussed in the context of precision diagnostics, targeted therapy and vaccine development. By elucidating pathogen adaptation dynamics and host-pathogen interactions, the present review offers a basis for reducing the global burden of drug-resistant infections through improved surveillance and personalized interventions.

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Correspondence to: Professor Shuyao Zhang or Dr Yun Chen, Department of Pharmacy, Guangzhou Red Cross Hospital (Guangzhou Red Cross Hospital of Jinan University), 396 Tongfu Zhong Road, Guangzhou, Guangdong 510220, P.R. China
E-mail: shuyao0754@qq.com
E-mail: gd_chenyun@163.com

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1. Introduction

Pathogens, including bacteria, viruses, fungi and parasites, are the primary causes of infectious disease in humans, animals and plants, posing a significant burden on global health and the environment. According to the World Health Organization, infectious diseases account for millions of deaths annually, leading to substantial economic losses due to increased healthcare costs, decreased labor productivity, and declines in agricultural and livestock production (1,2).

Virulence genes are key to the pathogenicity of pathogens and encode products that facilitate host infection, colonization, tissue invasion, immune evasion and disease symptomatology (3,4). Virulence genes dynamically co-evolve with antibiotic resistance genes in mobile genetic elements such as plasmids and transposons (5-13). This phenomenon was first identified in extended-spectrum β-lactamase (*ESBL*)-producing *Escherichia coli* strains, where adhesin (*fimH*) and β-lactamase *(blaCTX-M*) co-localize on incompatibility group F (*IncF*) plasmids (5). This is in contrast to earlier models that viewed virulence and resistance as independent genetic traits (6,14). In-depth exploration of virulence genes can accurately elucidate pathogenic mechanisms and provide key insights for the development of prevention and control strategies against infectious disease. Furthermore, such research aids the development of innovative diagnostic techniques, targeted therapeutic drugs and efficient vaccines, thereby promoting the advancement of precision medicine (4,15,16).

The pathogens discussed in the present review (such as *E. coli*, *S. aureus*, *C. albicans* and SARS-CoV-2) were selected based on three criteria: global disease burden, clinical relevance to antimicrobial resistance and utility as model organisms in virulence research (2,17). For example, *E. coli* is among the top causes of bacterial infection worldwide, with numerous annual cases of urinary tract infection and diarrheal diseases, whereas *ESBL*-producing strains exemplify the co-evolution of virulence and resistance genes on mobile genetic elements (1,5). *C. albicans*, responsible

for a large number of invasive fungal infections annually, serves as a model for studying fungal adaptability in immunocompromised hosts, such as biofilm-mediated resistance in catheter-associated infection (18). Emerging viruses such as SARS-CoV-2 have been prioritized because of their pandemic impact, with a large number of confirmed cases globally, highlighting the urgency of understanding virulence gene plasticity (such as *S* protein mutations driving immune evasion in o variants) (19). These criteria ensure a balanced representation of pathogens with notable public health implications and provide mechanistic insight into the virulence-antimicrobial resistance interplay.

The present review focuses on research progress in the field of pathogen virulence genes and practical applications. By integrating research findings on various pathogens, the characteristics, detection methods, and associations with drug resistance of different virulence genes can offer a reference for related research. This can also contribute to improving the clinical diagnosis and treatment of pathogenic infections and the prevention and control of drug resistance.

2. Mechanisms of pathogen virulence genes

Mechanisms of virulence genes in pathogens. The virulence genes of pathogens regulate infection processes through specific molecular mechanisms, exhibiting differences in the genetic basis, infection strategy, and environmental responses of bacteria, viruses and fungi. As detailed in Table I, virulence factors are functionally categorized into four classes based on their mechanisms: i) Toxin genes that disrupt host cell integrity; ii) Adhesin genes mediating pathogen attachment; iii) Invasion genes enabling barrier penetration; and iv) Immune evasion genes subverting host defenses, with representative examples across bacterial, viral and fungal pathogens. These genes collectively form a sophisticated pathogenic network.

Bacteria: Mobile genetic element-driven invasive and adaptive networks. Bacterial virulence genes often cluster within pathogenicity islands (such as the locus of enterocyte effacement island in *E. coli*) or mobile genetic elements, such as plasmids and transposons (17,20). CRISPR-CRISPR associated protein (Cas) systems, traditionally known for their role in bacterial immunity against bacteriophages, have been shown to regulate the expression of virulence genes (21). In *Salmonella*, Cas12a, a component of the CRISPR-Cas system, exhibits non-specific RNase activity when activated under host immune or antibiotic pressure (20,22). This activity leads to global transcriptional suppression and induces a dormant state in the bacterial population (22). This physiological shift decreases overall invasiveness [including expression of virulence factors such as those encoded by *Salmonella* pathogenicity island 1 (*SPI-1*)] and promotes bacterial persistence, representing a mechanism distinct from its canonical phage defense function and contributing to chronic infection dynamics (20,22).

During adhesion and colonization, the *fimH* gene of *E. coli* encodes the tip protein of type 1 pili that specifically binds to mannose receptors on urinary epithelial cells and serves as a key pathogenic factor in urinary tract infections (5,23). By contrast, the fibronectin-binding protein A (*fnbA)* gene of *Staphylococcus aureus* encodes fibronectin-binding proteins that mediate adhesion and colonization of damaged tissue surfaces (24,25). For invasion and immune evasion, enteropathogenic *E. coli* (EPEC) injects the effector protein translocated intimin receptor (Tir) into host cells via the type III secretion system [*T3SS*; enteropathogenic secretion genes *(esc)* gene cluster], inducing actin rearrangement to form 'pedestal' structures that disrupt the intestinal barrier (26,27). *Streptococcus pneumoniae* relies on the *capsular polysaccharide synthesis (cps)* gene cluster to synthesize capsular polysaccharides and evade complement-mediated phagocytosis by the host (28,29). In response to environmental stress within the host, the dormancy survival regulator *(dosR)* regulatory system of *Mycobacterium tuberculosis* is activated in the hypoxic microenvironment of macrophages, entering a dormant state by epigenetically silencing virulence genes (30). The polyketide synthase (*pks)* gene encodes polyketide synthase to synthesize wax D, which enhances resistance to lysosomal degradation (31,32). The capacity of these mobile genetic elements to carry multiple genes of these mobile genetic elements often leads to the colocalization of bacterial virulence and resistance genes, such as *fimH* and *blaCTX-M* co-carried on *IncF* plasmids in ESBL-producing *E. coli*, forming 'pathogenic-resistant' co-evolutionary modules (7).

The interaction between bacterial virulence genes and host immunity is a dynamic process that drives pathogen adaptation. For example, *M. tuberculosis* uses the *dosR* regulon, a hypoxia-responsive genetic network, to enter metabolic dormancy within macrophages, thereby evading immune system clearance. This regulon includes genes, such as *Rv3133c*, which are key for maintaining bacterial persistence in granulomas. However, host-derived IFN-γ disrupts *dosR*-mediated dormancy by suppressing its transcriptional activity, leading to bacterial reactivation and the release of virulence factors such as early secreted antigenic target 6 kDa, which triggers granuloma rupture and systemic dissemination (33,34). Similarly, in ESBL-producing *E. coli*, selective pressure from antibiotic use promotes co-localization of virulence genes (such as *fimH*, encoding type 1 fimbriae) and resistance genes (such as *blaCTX-M*) on conjugative plasmids (7). Clinical data have revealed that *fimH* is more prevalent in ESBL-producing strains compared with non-ESBL-producing strains, with co-localized plasmids exhibiting markedly enhanced HGT efficiency (5). This highlights how host-imposed antibiotic pressure directly facilitates the spread of hypervirulent resistant clones in the gastrointestinal tract. Additionally, *S. aureus* dynamically modulates biofilm formation via the intracellular adhesion locus ABCD (*icaABCD*) operon under neutrophil attack. Exposure to neutrophil-derived reactive oxygen species (ROS) upregulates ica expression, leading to biofilm structures that substantially decrease phagocytic clearance and are associated with increased bacterial persistence in chronic infection (35,36). These findings illustrate how bacterial pathogens exploit host immune cues to recalibrate their virulence strategies.

Viruses: Host-dependent invasion and antigenic variation strategies. Viral virulence genes directly determine infection tropism and immune escape efficiency, with functions that are dependent on host cell receptors and signaling pathways (37,38). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein binds to lung cells via its

Table I. Functional classification of virulence genes in bacterial, viral and fungal pathogens.

Authors, year	Category	Function	Examples	(Refs.)
Goldsmith *et al*, 2023; Gerding *et al*, 2014	Toxin genes	Encode toxins that disrupt host cell integrity and physiological function or induce tissue damage	*ctxAB* of *Vibrio cholerae* causes severe diarrhea and electrolyte imbalance; botulinum toxin of *Clostridium botulinum* is a neurotoxin blocking neurotransmitter release; *TcdA* and *TcdB* of *Clostridioides difficile* are enterotoxins inducing colonic inflammation and epithelial damage	(178,184)
Ullah *et al*, 2023; Speziale *et al*, 2020; Bing *et al*, 2023	Adhesin genes	Mediate pathogen attachment to host cell receptors, facilitating colonization and invasion	*fimH* of *Escherichia coli* is a type 1 fimbrial tip protein that binds urinary epithelial mannose receptors; *fnbA* and *fnbB* of *Staphylococcus aureus* are fibronectin-binding proteins that enhance tissue adhesion; *ALS3* of *Candida auris* is a hyphal adhesin that promotes biofilm formation on medical devices	(5,24,74)
Watanabe *et al*, 2024; Yang *et al*, 2023	Invasion genes	Enable pathogens to breach host barriers and invade tissues or cells	*ipaBCD* of *Shigella* encodes *T3SS* effectors (*ipaB*, *ipaC*, *ipaD*) that mediate both adhesion to and invasion of intestinal epithelial cells.; *inlA* and *inlB* of *Listeria monocytogenes* are internalins mediating host cell entry via E-cadherin; *T3SS* (*esc* cluster) of enteropathogenic *Escherichia coli* injects effector Tir to induce actin pedestal formation	(94,27)
Chun *et al*, 2023; Liang *et al*, 2024; Lin *et al*, 2023	Immune evasion genes	Interfere with immune recognition, suppress host defense or evade clearance mechanisms	In *Streptococcus pneumoniae*, the capsular polysaccharide synthesis gene inhibits phagocytosis; UL49.5 of herpes viruses blocks antigen presentation by host MHC-I; EFG1 of *Candida albicans* regulates hyphal transition and *SAP* protease secretion to degrade host antimicrobial peptides	(28,54,55)

ctxAB, *cholera* toxin A-B subunit; Tcd, *Clostridioides difficile* toxins; *fimH*, type 1 fimbrial adhesin; fnb, fibronectin-binding protein; *ALS3*, agglutinin-like sequence 3; *ipaBCD*, invasion plasmid antigens B, C and D; T3SS, type III secretion system; inl, internalin; *esc*, enteropathogenic secretion genes; Tir, translocated intimin receptor; UL49.5, herpesvirus UL49.5 protein; MHC-I, major histocompatibility complex class I; Efg, enhanced filamentous growth protein; SAP, secreted aspartyl protease.

receptor-binding domain (RBD). The N501Y mutation of the o variant emerged under antibody pressure, driven by template switching during replication, a mechanism uncovered through single-genome sequencing (39). This finding contrasts with earlier hypotheses that viral mutations are randomly distributed (40). The human immunodeficiency virus *(HIV) envelope (env) gene* encodes glycoprotein (gp)120, which binds to $CD4^+$ T cell receptors to mediate viral membrane fusion with host cell membranes (41-45). To escape host immunity, the hemagglutinin (*HA*) gene of influenza viruses undergoes antigenic drift (such as point mutations in the HA1 domain of H3N2), altering the antibody recognition sites (37,46). The Hepatitis B virus X protein (HBx) protein of the hepatitis B virus interferes with host DNA repair and apoptosis signals by inhibiting the p53 pathway (45,47). In terms of gene expression regulation, adenovirus early region 1A activates host cell cyclins to create conditions for viral DNA replication (48,49). The quasispecies diversity of viruses (such as the high mutation rate of HIV) leads to the dynamic evolution of virulence phenotypes, making monotherapy prone to inducing drug-resistant mutations (50,51).

Viral evolution is associated with host immune surveillance. The SARS-CoV-2 S protein RBD undergoes mutations (such as o N501Y) under neutralizing antibody pressure, enhancing angiotensin-converting enzyme (ACE)2 affinity while evading antibody recognition (19). HIV-1 exhibits a similar plasticity. Hypermutation of the *env* gene in regions targeted by $CD8^+$ T cells drives immune escape, with notably higher mutation rates in patients with robust cytotoxic T lymphocyte response (52). Additionally, prolonged type I IFN exposure results in the selection of influenza A virus mutants [such as polymerase basic 2 (*PB2*) E627K] that evade IFN-mediated restriction, enhancing their replication in human cells (53). These examples highlight the evolutionary race between viral adaptation and host immunity.

Fungi: Dual virulence networks of morphological transition and microenvironmental response. Fungal virulence gene expression is regulated by the host microenvironment (temperature, pH and nutrients), facilitating invasion and colonization through morphological plasticity and metabolic adaptation (16,54). In *Candida albicans*, enhanced filamentous growth protein 1 (EFG1) triggers yeast-to-hyphal growth at body temperature (16). Recent studies have shown that low pH in macrophage phagosomes activates histone deacetylase 1 (Hda1), an enzyme that modifies the EFG1 promoter via histone H3 lysine 9 acetylation marks, linking environmental cues to virulence (16,55). *This mechanism has not been identified in previous transcriptional profiling studies* (56,57). Hyphal wall protein 1 *(HWP1)* expresses hyphal wall proteins that mediate host cell adhesion and tissue penetration (54,57). The capsule induction gene 1 (*Cig1*) gene of *Cryptococcus neoformans* regulates capsular polysaccharide synthesis, notably increasing capsule thickness in macrophages (58,59). During immune evasion and damage, the rodlet protein A (*RodA*) hydrophobic protein of *Aspergillus fumigatus* mediates adhesion of conidia to the respiratory epithelium (60,61). The stress seventy subfamily A member 1 (*ssa1)* gene of *Candida auris* regulates heat shock protein (HSP) expression to enhance survival in hospital environments while carrying the azole resistance gene ergosterol biosynthesis gene 11 (*ERG11*) (62). Additionally, the biofilm and cell wall regulator 1 (*Bcr1*) gene of *C. albicans* regulates biofilm matrix synthesis, substantially decreasing antifungal drug penetration efficiency and leading to refractory catheter-associated infections (63-66).

Fungal pathogens exhibit adaptability to the host immune microenvironment. *C. albicans* transitions from yeast to hyphae in response to macrophage-derived reactive oxygen species (ROS), a process governed by the transcription factor EFG1. Hyphal morphogenesis enables tissue penetration and is coupled with the secretion of aspartyl proteases (*Sap2-6*), which degrade host antimicrobial peptides such as defensins and markedly decrease phagocytic killing (55,67). In immunocompromised hosts, *C. albicans* exploits IL-17A deficiency to colonize mucosal surfaces, with IL-17A-knockout mice showing a substantially higher fungal burden compared with wild-type controls (68). *A. fumigatus* adapts to hypoxic lung environments by upregulating iron acquisition genes siderophore biosynthesis enzyme A (*SidA*) and the allergenic protease aspf1 (Asp f1). Hypoxia-inducible factor 1α signaling in host epithelial cells exacerbates fungal virulence by promoting *SidA*-mediated iron scavenging, and enhancing fungal survival and tissue invasion (69-71). These mechanisms highlight the bidirectional interaction between fungal virulence and the host immune defense.

Comparative mechanisms of virulence across pathogen types. Although bacteria, viruses and fungi employ distinct virulence strategies, shared themes emerge in their adaptations to the host environment. Bacterial pathogens (such as *E. coli* and *S. aureus*) often use secretion systems (such as *T3SS*) to directly inject effector proteins into host cells, thereby disrupting cellular processes (36,72). By contrast, viral pathogens (such as SARS-CoV-2 and HIV) rely on the host machinery for replication, with virulence genes (such as S protein and *env*) mediating entry and immune evasion through rapid mutations (19,39). Fungal pathogens (such as *C. albicans* and *A. fumigatus*) exhibit morphological plasticity (such as the yeast-to-hyphal transition) and secrete hydrolases to penetrate host barriers (55,73). A unifying feature of all pathogens is the dynamic regulation of virulence genes in response to host immune pressure, such as biofilm formation in bacteria and antigenic drift in viruses (19,29) (Table II).

Comparative mechanisms of immune-driven virulence evolution. Host immune pressure exerts selective forces on pathogen evolution, driving adaptations that balance pathogen survival and virulence. *C. auris* exemplifies this dynamic through its unique stress response strategy (62,74). When challenged by neutrophil-derived ROS, *C. auris* activates the *HSP90*-calcineurin axis, a highly conserved pathway (62). *HSP90*, a molecular chaperone, stabilizes calcineurin under oxidative stress, enabling the fungus to maintain hyphal morphogenesis and escape phagolysosomal degradation (62,74). Pharmacological inhibition of *HSP90* with geldanamycin disrupts this axis, restoring fluconazole susceptibility in resistant clinical isolates (62). This contrasts with *C. albicans*, which predominantly relies on the *EFG1*-mediated pathway for hyphal transition under similar conditions (16,55). Notably, *C. auris* exhibits higher *HSP90* expression in host niches than *C. albicans*, a trait amplified in multidrug-resistant strains (62,74). Transcriptomic profiling has revealed that *C. auris* integrates *HSP90* activity with up-regulation of the

Table II. Mechanisms of action of virulence genes in pathogens.

A, Bacteria

Authors, year	Virulence gene	Mechanism	Impact on host cells	Impact on the intracellular environment	Impact on host tissues	Immune evasion strategy	Host immune driver	(Refs.)
Yang *et al*, 2023; Schnappinger *et al*, 2003; Rahlwes *et al*, 2023	EPEC *esc/eae*	*T3SS* injects effectors (such as *Tir*), inducing actin rearrangement and pedestal formation	Cytoskeletal disruption and tight junction damage	Hijacks host signaling pathways (Rho GTPases)	Disrupts intestinal barrier, causing diarrhea	Molecular mimicry of host signals	TNF-α/IFN-γ from mucosal inflammation triggers effector secretion	(27,33,86)
Korshoj *et al*, 2024; Peng *et al*, 2022; Li *et al*, 2024	*Staphylococcus aureus agr/ ica*	*agr* quorum sensing regulate toxin (*hla*) and biofilm (*icaABCD*) expression; *ica* synthesizes biofilm matrix	α-hemolysin lyses erythrocytes; biofilm creates physical barrier	Metabolic dormancy in biofilm niches; ROS resistance	Skin necrosis; chronic implant infection	Biofilm blocks phagocyte penetration; *PVL* lyses neutrophils	Neutrophil-derived ROS upregulate *ica* expression	(29,35,101)
Cheng *et al*, 2024; Gao *et al*, 2024	*Mycobacterium tuberculosis dosR/pks*	*dosR* induces dormancy in hypoxia; *pks* synthesizes cell wall lipids, resisting degradation	Inhibits phagolysosome fusion; alters antigen presentation	Epigenetic silencing of host pro-inflammatory genes	Granuloma formation; lung cavitation	Dormancy avoids immune detection; wax D resists lysosomal enzymes	IFN-γ suppresses *dosR*, reactivating infection	(30,31)

B, Viruses

Authors, year	Virulence gene	Mechanism	Impact on host cells	Impact on the intracellular environment	Impact on host tissues	Immune evasion strategy	Host immune driver	(Refs.)
Addetia *et al*, 2023; Suzuki *et al*, 2022; Bdeir *et al*, 2025	SARS-CoV-2 S/ *ORF3a*	S protein (RBD) binds ACE2; *ORF3a* disrupts ion channels and induces apoptosis	Membrane fusion enables viral entry; mitochondrial dysfunction	ER stress; inflammasome activation	Diffuse alveolar damage; multiorgan failure	S mutation (such as o N501Y) evade neutralizing antibodies	Antibody pressure drives RBD antigenic evolution	(19,131,133)
Ding *et al*, 2024; Lederhofer *et al*, 2024;	Influenza *HA/PB2*	*HA* mediates receptor binding; *PB2* mutations	Receptor-mediated endocytosis	Altered viral RNA synthesis kinetics	Respiratory epithelial destruction;	*HA1* domain mutations cause antigenic drift	Seasonal vaccine pressure selects *HA* mutants	(53,137, 140)

Table II. Continued.

B, Viruses

Authors, year	Virulence gene	Mechanism	Impact on host cells	Impact on the intracellular environment	Impact on host tissues	Immune evasion strategy	Host immune driver	(Refs.)
Zhang *et al*, 2024		enhance polymerase activity in mammalian cells			viral pneumonia			
Zhang *et al*, 2023; Fu *et al*, 2024; Li *et al*, 2024	HIV *env/tat*	*Env* encodes gp120/gp41 for $CD4^+$ binding; *tat* transactivates viral transcription	Membrane fusion; $CD4^+$ T cell depletion	Disrupts NF-κB signaling; inhibits DNA repair	Lymphoid tissue destruction; AIDS progression	*Env* hypermutation in V3 loop evades neutralizing antibodies	$CD8^+$/T cell pressure drives *env* diversification	(41,43,52)

C, Fungi

Authors, year	Virulence gene	Mechanism	Impact on host cells	Impact on the intracellular environment	Impact on host tissues	Immune evasion strategy	Host immune driver	(Refs.)
Liang *et al*, 2024; Zhang *et al*, 2024	*Candida albicans* EFG1/*SAP*	EFG1 triggers hyphal transition at 37˚C; *SAP* proteases degrade host defensins	Hyphal penetration of epithelial barriers	*Sap2-6* degrade antimicrobial peptides in phagolysosomes	Mucosal thrush; disseminated candidiasis	Hyphae resist phagocytosis; cell wall mannan masking	Macrophage ROS induce EFG1-mediated morphogenesis	(54,57)
Carrion *et al*, 2013; Hoang *et al*, 2022; Liu *et al*, 2023	*Aspergillus fumigatus RodA/SidA*	*RodA* mediates conidial adhesion; *SidA* acquires iron under hypoxia	Epithelial damage; endothelial invasion	HIF-1α-mediated iron scavenging enhances survival	Tissue-invasive aspergillosis; hemorrhagic necrosis	Melanin shields antigens; gliotoxin induces immune cell apoptosis	Hypoxia upregulates *SidA* via host epithelial HIF-1α	(61,69,70)
Kim *et al*, 2019; Tian *et al*, 2024; Muñoz *et al*, 2018	*Candida auris ssa1/ERG11*	*ssa1* (HSP90) enables thermotolerance; *ERG11* mutations confer azole resistance	Enhanced biofilm formation on medical devices	*Ssa1* stabilizes efflux pumps (*CDR1*); biofilm decreases drug penetration	Persistent catheter-associated infection	*HSP90*-calcineurin axis promotes hyphal escape from phagolysosomes	Neutrophil ROS activate the *HSP90*-calcineurin stress response	(62,162,164)

EPEC, enteropathogenic *E. coli*; *esc*, enteropathogenic secretion genes; *eae*, *E. coli* attaching and effacing; T3SS, type III secretion system; Tir, translocated intimin receptor; agr, accessory gene regulator; *icaABCD*, intracellular adhesion locus ABCD; *hla*, α-hemolysin; ROS, reactive oxygen species; PVL, Panton-Valentine leukocidin; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; S mutations, Spike protein mutations; RBD, receptor-binding domain; *ORF3a*, Open Reading Frame 3a; ER, endoplasmic reticulum; *HA*, hemagglutinin; *PB2*, polymerase basic 2; HIV, human immunodeficiency virus; *env*, envelope gene; *tat*, transactivator of transcription; gp, glycoprotein; AIDS, acquired immune deficiency syndrome; Efg, enhanced filamentous growth protein; SAP, secreted aspartyl protease; *RodA*, rodlet protein A; *SidA*, siderophore biosynthesis enzyme A; *ssa1*, stress seventy subfamily A member 1; ERG, ergosterol biosynthesis gene; HSP, heat shock protein; CDR, Candida drug resistance; ACE2, angiotensin-converting enzyme 2; *dosR*, dormancy survival regulator; HIF-1α, hypoxia-inducible factor 1α; *pks*, polyketide synthase; *S/ORF3a*, spike protein/Open Reading Frame 3a.

efflux pump gene Candida drug resistance 1 (*CDR1*), thereby establishing a dual defense against immune clearance and antifungal drugs (62). Such adaptations explain its dominance in immunocompromised hosts, where simultaneous immune evasion and drug resistance confer survival advantages.

These evolutionary adaptations mirror the strategies observed in *A. fumigatus*, where hypoxia-induced *HSP90* upregulation enhances iron acquisition and tissue invasion (73). *C. auris* uniquely co-opts this pathway to bridge environmental persistence and clinical virulence. For example, *HSP90*-dependent calcineurin signaling not only promotes hyphal growth but also activates biofilm-associated adhesins agglutinin-like sequence 3 (*ALS3*), facilitating the colonization of medical devices (74). This multifunctional exploitation of stress-response pathways highlights the ability to thrive under diverse selective pressures, from hospital disinfection protocols to host immune responses.

Evolutionary dynamics of virulence genes

Horizontal gene transfer (HGT) and virulence dissemination. Bacterial virulence evolution is shaped by HGT, a process driven by mobile genetic elements such as plasmids, transposons and pathogenicity islands, which enable the rapid dissemination of virulence factors and typically couple virulence with antibiotic resistance (7,10). In *E. coli*, the *IncF* plasmid exemplifies this phenomenon by co-localizing the fimH adhesin gene (key for urinary tract infection) and the blaCTX-M β-lactamase gene, resulting in ESBL-producing strains with higher fimH prevalence, with notably enhanced transfer efficiency (8,9). The transposon 554 (Tn554) transposon in *S. aureus* carries both the erythromycin resistance methylase (erm) resistance gene and the exfoliative toxin B (etb) toxin gene to drive multidrug-resistant, hypervirulent clones, such as USA300, which exhibit markedly higher transfer efficiency and mortality rates in severe infection (75). CRISPR-Cas systems, which are typically linked to phage defense, participate in the evolution of virulence. Under immune pressure, *Salmonella* Cas12a cleaves transcripts of the SPI-1 virulence island (21). This downregulates invasion genes to balance the severity of infection with host survival and promotes chronicity of the disease.

Immune pressure-driven genetic adaptation. Host immune responses impose selective pressure on viral virulence determinants, driving adaptive genetic diversification and structural evolution (37,53). Viruses such as influenza A and SARS-CoV-2 exhibit accelerated antigenic evolution through continuous genomic alterations (37,53). Seasonal H3N2 strains accumulate mutations in the HA gp (such as the E190G substitution) that compromise the antibody neutralization capacity and diminish vaccine effectiveness (37,46). Concurrently, the SARS-CoV-2 o variant exhibits key amino acid substitutions in the RBD of the S protein, notably the N501Y mutation, resulting in enhanced ACE2 receptor binding affinity alongside immune evasion from neutralizing antibodies (19,53). Bacteria such as *S. pneumoniae* modify *cps* genes to alter the capsular polysaccharide structure, notably decrease complement deposition and evade vaccine-induced immune responses (75). *Shigella* uses *T3SS* to inject invasion plasmid antigen B (*ipaB*), a protein that mimics host apoptotic signals, to suppress inflammatory IL-1β secretion (76). Fungi such as *C. albicans* use epigenetic reprogramming, in which the Hda1 enzyme modifies the EFG1 promoter to activate hyphal transition, enabling hyphae to secrete *Sap2-6* proteases that degrade host defensins and decrease the phagocytic killing efficiency (55).

Morphological and metabolic evolution in fungal pathogens. Fungal virulence genes have evolved to optimize adaptation to host microenvironments through morphological plasticity and metabolic reprogramming. *C. albicans* undergoes yeast-to-hyphal transition at 37˚C, driven by EFG1-mediated activation of *HWP1* and other hyphal genes, which enhances tissue invasion and biofilm formation via *Bcr1*-regulated exopolysaccharides that decrease drug penetration (54). *C. auris* links virulence and drug resistance through genetic colocalization of *ssa1* (encoding a heat shock protein, HSP70) and *ERG11* (encoding lanosterol 14α-demethylase, a target of azole antifungals), allowing survival in hospital environments with high heat stability and decreased fluconazole binding due to ERG11 mutations (74,77,78). In hypoxic host niches, *A. fumigatus* upregulates *SidA* for iron acquisition via siderophores, a process enhanced by host HIF-1α signaling. *RodA* hydrophobins on conidia shield antigens to decrease dendritic cell activation (57,73).

Cross-kingdom convergence in virulence evolution. Pathogens across kingdoms exhibit convergent evolutionary strategies to overcome host defenses, as evidenced by their genetic plasticity, immune evasion and microenvironmental adaptations. Bacteria, viruses and fungi prioritize immune evasion: Bacteria form biofilms (such as *Pseudomonas aeruginosa* Pellicle polysaccharide synthesis locus), viruses mutate surface proteins (such as SARS-CoV-2 *S*) and fungi remodel cell walls (such as *Cryptococcus* capsules) (79). Microenvironmental adaptation is equally universal among pathogens. *M. tuberculosis* activates the *dosR* regulon to establish dormancy within hypoxic granulomas, *A. fumigatus* up-regulates the siderophore synthase gene *SidA* for iron acquisition in oxygen-poor pulmonary niches, and *influenza A virus* acquires the PB2-E627K mutation to optimize polymerase function at mammalian body temperature (30,53,70). These convergent strategies reflect a shared evolutionary imperative to balance survival, replication and transmission within the constraints of the host immunity and physiology. Based on their functions and mechanisms of action, virulence genes can be classified into categories (Fig. 1).

Research challenges and unresolved questions. Despite these significant advances, several critical gaps remain in the understanding of the biology underlying virulence genes.

Unclear molecular mechanisms of cross-species virulence gene HGT. HGT of virulence genes is documented in bacteria (such as *E. coli* plasmids carrying *fimH-blaCTX-M*) (5), but interkingdom HGT between fungi and bacteria remains mechanistically elusive. For *Cryptococcus neoformans*, the Capsule-Associated Protein (*CAP*) cluster encoding its polysaccharide capsule (a key virulence factor) shows phylogenetic clustering with glycosyltransferase genes from soil *Streptomyces Spp* supported by transposase-flanked insertion signatures in the fungal genome (80). Functional assays

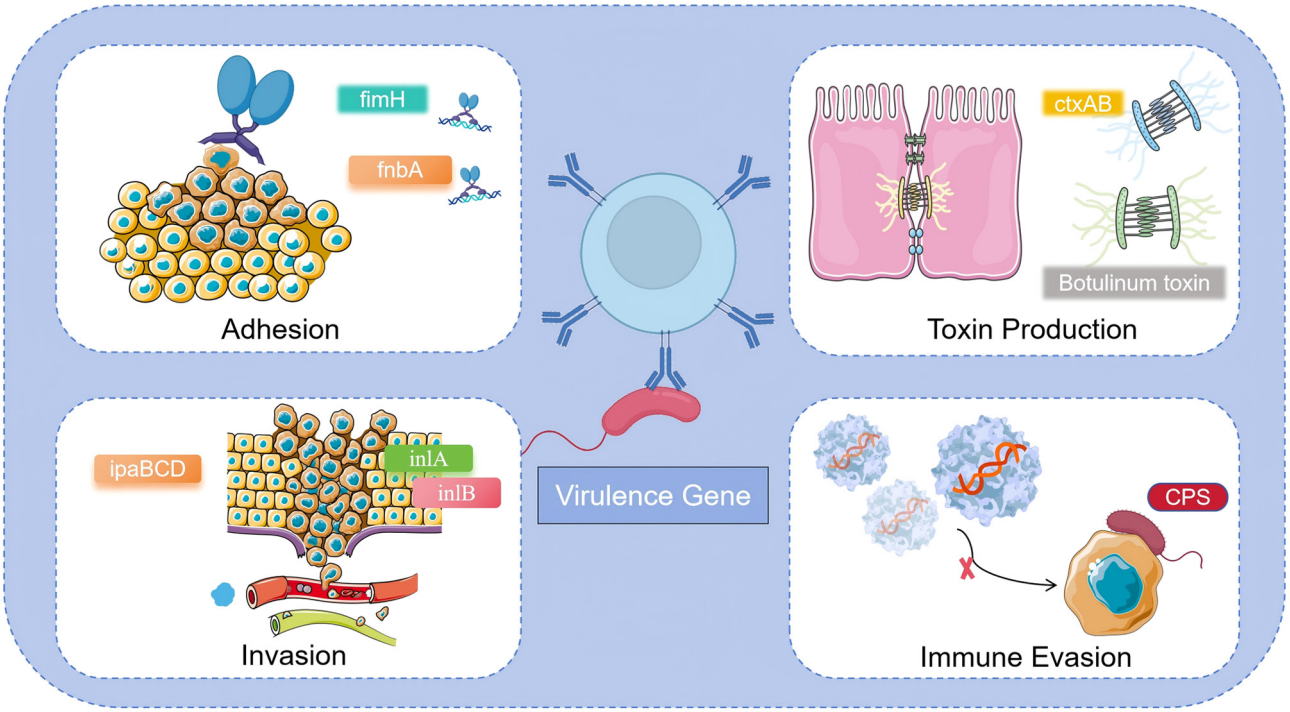


Figure 1. Mechanisms of action of various virulence genes. FimH (type 1 fimbrial adhesin), FnbA (fibronectin-binding protein A), IpaBCD (invasion plasmid antigens B, C and D), CtxAB (cholera toxin subunits A and B), CPS (capsular polysaccharide synthesis locus), and In1 (internalin-like invasion factor). FimH mediates mannose-based adhesion of *E. coli* to uroepithelium; FnbA anchors *Staphylococcus aureus* to fibronectin for tissue invasion; IpaBCD drives *Shigella* entry via actin remodeling; CtxAB triggers chloride/water efflux causing diarrhea; Botulinum toxin blocks acetylcholine release producing flaccid paralysis; InlA/InlB enable *Listeria* to cross intestinal epithelia; CPS forms *pneumococcal* capsules that resist phagocytosis; together they exemplify adhesion, invasion, immune evasion and toxin production.

have further demonstrated that heterologous expression of *S. coelicolor* glycosyltransferase gene *(Sc_GT)* complements capsule defects in *C. neoformans CAP64* mutants, restoring macrophage survival and virulence in murine models (80-82). These findings align with broader patterns of fungal-bacterial HGT in soil ecosystems, where predation (such as fungal trapping of bacteria) and plasmid-mediated transfer facilitate genetic exchange (81). However, eukaryotic barriers, such as nuclear envelope sequestration and intron-exon splicing incompatibilities, may limit such transfer, explaining why a small proportion of fungal virulence genes show interkingdom origins (16,79). Recent studies have highlighted the potential role of ecological associations in facilitating HGT (79,80,82). For example, soil environments, where fungi and bacteria coexist, may promote genetic exchange through shared niches or phagotrophic interaction. However, experimental validation of such transfers is challenging due to the complexity of replicating natural soil microbiomes *in vitro* and the scarcity of definitive genetic markers for recent cross-kingdom HGT events (79,81). Phylogenetic analyses of *C. neoformans* capsule genes (such as *CAP59* and *CAP60*) have revealed limited similarity to bacterial homologs, suggesting either ancient transfer events or alternative evolutionary pathways (80,81). Additionally, the lack of conserved synteny or horizontal transfer signatures (such as atypical GC content) further complicates the identification of bacterial donors (79).

Another key gap is the paucity of mechanistic insight into how bacterial genetic material integrates into fungal genomes. While HGT in prokaryotes commonly occurs via plasmids, phages or transposons, analogous mechanisms in fungi are less characterized (10). For example, transposon-mediated gene transfer has been observed in *Cryptococcus* during infection, but its role in acquiring bacterial-derived virulence genes remains speculative (82). Furthermore, the functional expression of horizontally transferred genes in fungi requires compatibility with eukaryotic transcriptional and translational machinery, which may limit successful transfer (83).

To address these gaps, recent studies have leveraged comparative genomics and experimental evolution (82,83). For example, genome-wide surveys of fungal pathogens such as *Colletotrichum* have identified bacterial-derived genes involved in carbohydrate metabolism and cell wall degradation, suggesting HGT contributes to niche adaptation (81,84). Similarly, experimental co-culture systems have demonstrated interspecific chromosome transfer in fungi, although these events primarily involve fungal-fungal interactions (83,84).

Spatiotemporal heterogeneity in host microenvironment-driven virulence gene regulation. Models of virulence gene regulation typically overlook spatiotemporal variations in host tissue (30,33,34). For example, *M. tuberculosis* in hypoxic granuloma activates the *dosR* regulon. However, the differential impact of macrophage polarization (M1/M2 phenotypes) on *dosR* expression across infection foci remains unclear (33,34). *dosR* regulon is key for *M. tuberculosis* to adapt to the hypoxic environment within granuloma. It allows the bacterium to enter a dormant state, thereby evading host immune surveillance and increasing its chances of survival (30,33,34,85,86). This highlights the complexity of host-pathogen interactions and the need

for more precise models that account for microenvironmental heterogeneity (30,33,34,86).

Recent studies have investigated the dynamics of macrophage polarization and its effect on *dosR* expression (30,34,87). For example, in the early stages of infection, macrophages are predominantly of the M1 phenotype, which is characterized by the production of pro-inflammatory cytokines such as TNF-α and IFN-γ. These cytokines may suppress *dosR* expression, enabling *M. tuberculosis* to maintain higher metabolic activity and virulence, facilitating its rapid dissemination within the host (30,34). As the infection progresses and the inflammatory response persists, some macrophages transition to the M2 phenotype, which is associated with anti-inflammatory cytokines such as IL-10. This shift may create a more favorable environment for *M. tuberculosis* to activate its *dosR* regulon (34,87). The activation of *dosR* allows the bacterium to enter a dormant state, enhancing its resistance to host immune attacks and improving its chances of long-term persistence within the host (30).

In addition to macrophage polarization, other microenvironmental factors also serve a role in the regulation of *dosR* expression. For example, the availability of nutrients and the presence of other immune cells influence the metabolic state of *M. tuberculosis* (31). These findings underscore the need for a more nuanced understanding of how host microenvironments dynamically interact with bacterial virulence genes. Such insights may facilitate development of novel therapeutic strategies that target these regulatory mechanisms, potentially improving the efficacy of treatment for tuberculosis and other chronic infectious diseases.

Lack of understanding of cross-kingdom regulation of virulence genes by commensal microbiota. To the best of our knowledge, the influence of commensal microbiota on the expression of virulence genes remains unclear. For example, short-chain fatty acids (SCFAs) produced by gut bacteria may modulate *E. coli fimH* expression. However, direct experimental evidence linking microbiota metabolites to virulence activation is lacking (88). Recent studies have shown that SCFAs, primarily acetate, propionate and butyrate, serve a significant role in regulating the growth and virulence of enteric pathogens such as *E. coli* (89-91). In the ileum, where SCFA concentrations are relatively low, SCFAs enhance the growth and motility of pathosymbiont *E. coli*. However, in the colon, where SCFA concentrations are higher, SCFAs inhibit the growth of *E. coli* and downregulate the expression levels of virulence genes such as *fimH*, flagellin C gene (*fliC*), high-temperature requirement A gene (*htrA*), hemoglobin uptake receptor A gene (*chuA)* and *pks*. This downregulation is associated with decreased bacterial motility, infectivity and type 1 fimbria-mediated agglutination. SCFAs also inhibit the activation of NF-κB and the production of IL-8 by epithelial cells, suggesting a role in modulating host inflammatory responses (90).

SCFAs differentially regulate virulence genes in *E. coli* depending on their concentration and the pH of the environment. In a study evaluating the effect of SCFAs on virulence gene expression, colonic SCFAs downregulated the expression of virulence genes in a concentration-dependent manner (89,90). Specifically, genes involved in adhesion (*fimH*), motility (*fliC*), invasion (invasion plasmid antigen H) and biofilm formation (Biofilm stimulating substance S, *BssS*) were downregulated in the presence of higher concentrations of SCFAs. Conversely, ileal SCFAs upregulated these genes. This suggests that the chemical microenvironment of the gut, including SCFA levels and pH, serves a key role in determining the virulence potential of *E. coli* (88).

In addition to SCFAs, other metabolites produced by commensal microbiota may influence the expression of virulence genes. For example, quorum-sensing molecules such as acyl-homoserine lactones (AHLs) have been detected in the gut microbiota and regulate the expression of virulence genes in pathogens such as *P. aeruginosa* and *Salmonella* (91,92). Although the production of AHLs by commensal bacteria is not fully understood, the presence of (Luminescence Regulator homologs in numerous gut bacteria suggests that they can respond to AHLs produced by other members of the microbiota (92). This cross-kingdom signaling may provide a mechanism for pathogens to sense and adapt to the presence of commensal bacteria, thereby modulating their virulence gene expression. Overall, while there is growing evidence that commensal microbiota and their metabolites modulate the virulence of pathogens, the exact mechanisms and the full extent of these interactions remain to be elucidated (92). Further research is needed to understand the complex regulatory networks involved in cross-kingdom regulation of virulence genes, which may provide novel therapeutic strategies to prevent and treat infections (92).

3. Virulence genes in different pathogens

Typical cases of bacterial pathogens

E. coli. *E. coli* is a common flora in the intestinal tract, and certain strains carry virulence genes that have become important pathogens, causing intestinal infection and various diseases (5). In recent years, the production of *ESBL*s in *E. coli* has received considerable attention, and the association between resistance and virulence has become a major research focus (5,7,89). A study on clinical isolates of *ESBL*-producing and non-*ESBL*-producing *E. coli* showed significant differences in the distribution of virulence genes (5). When producing *ESBL*s strains, common virulence genes such as *fimH* (encoding type I fimbriae, mediating bacterial adhesion to host cells), pyelonephritis-associated pilus assembly protein C [associated with P pilus synthesis (where P denotes pyelonephritis), enhancing the adhesion of epithelial cells of bacteria in the urethra] and α-hemolysin (*hla*; encoding hemolysin, with a host cell membrane damage detection rate higher than that in non-ESBL-producing strains) were observed. Compared with *ESBL*-producing strains, the virulence gene carry rate in non-*ESBL*-producing strains is relatively low (5,23,93). Further studies have found that the drug resistance and virulence genes of *ESBL*-producing *E. coli* are typically located on the same plasmid, suggesting that drug resistance and virulence may co-evolve at the plasmid level, so the bacteria can still maintain strong pathogenicity while facing the pressure of antibiotics, which brings challenges for clinical treatment (7,89).

The *T3SS*, a needle-like structure in *EPEC*, injects effector proteins (such as *Tir*) into host intestinal cells (26,94). These effectors hijack host signaling pathways, inducing actin rearrangement and forming pedestal-like structures that anchor

bacteria to the epithelium, disrupting the barrier function and causing diarrhea (27,95). For example, the *esc* gene cluster of EPEC encodes structural proteins of *T3SS*, and the *E. coli* attaching and effacing gene encodes intimin, an outer membrane adhesin that mediates tight bacterial attachment to host intestinal cells. These virulence genes work together to promote tight adherence of bacteria to the surface of intestinal epithelial cells, trigger cytoskeletal rearrangement and form characteristic base structures that disrupt intestinal barrier function and cause intestinal infectious symptoms such as diarrhea and abdominal pain (26,94,95). In addition, recent studies have found that virulence genes carried by certain *E. coli* strains sense changes in the intestinal environment, such as changes in nutrient concentration, pH and composition of the intestinal microbial community, and dynamically regulate the expression of virulence genes (Table III) (27,95). When the intestinal environment changes, such as due to a decline in host immunity or a change in dietary structure, these virulence genes are activated, which converts the relatively harmless commensal bacteria into pathogenic bacteria and causes infection (27,96,97).

S. aureus is a common pathogenic bacterium in clinical practice that causes serious infectious diseases, including skin and soft tissue infections, pneumonia and endocarditis (Table III). Studies on clinical isolates have revealed that the virulence genes of *S. aureus* are diverse and governed by complex regulatory networks (98-100). For example, the accessory gene regulator (*agr*) locus is a key regulatory system for the expression of virulence genes in *S. aureus*. It comprises *agrA*, *agrB*, *agrC* and *agrD*. The *agr* system can detect changes in bacterial population density. When the bacterial population reaches a certain threshold, the autoinducible peptide encoded by the *agrD* gene is secreted and accumulates, and binds the *agrC* receptor and activates the *agrA* protein, thereby regulating the expression of downstream virulence genes. In the early stages of infection, when the number of bacteria is low, the *agr* system is in a low-activity state, which is conducive to bacterial adhesion and colonization, and the expression levels of surface adhesion protein genes (*fnbA* and clumping factor A) are upregulated. With the progression of infection, bacterial proliferation activates the agr system, enhancing expression of toxin genes including *hla* and δ-hemolysin (encoding pore-forming hemolysins), and *sea* and *seb* (encoding the superantigenic staphylococcal enterotoxins A and B, respectively, which induce vomiting and diarrhea). Bacteria release a large number of toxins during adhesion, invasion and diffusion, causing serious damage to host tissue (98,101,102).

The ability of *S. aureus* to form biofilms, in which the *ica* gene cluster serves a key role, is an important factor in its pathogenicity. Genes such as *icaA* and *icaD* encode products involved in the synthesis of exopolysaccharides, the primary matrix components of biofilms (103-105). Biofilms not only provide a physical protective barrier for bacteria to resist the killing of antibiotics and phagocytosis of host immune cells but also enhance the ability of bacteria to adhere to the surface of medical devices (such as catheters and artificial joints), leading to the occurrence of chronic infection and increasing the difficulty and cost of treatment (103-105). For example, in cases of infection associated with implanted medical devices, following the formation of an *S. aureus* biofilm, it is difficult to completely eliminate the bacteria using conventional antibiotic treatment, and the implants often need to be removed to control the infection, which causes pain and economic burden to patients (100). In addition, expression of virulence genes in *S. aureus* is regulated by internal environmental signals (such as cytokines secreted by host cells and local oxygen concentration) (99,101,106). Bacteria adjust virulence strategies according to their microenvironment to adapt to different stages of the infection process, highlighting the complexity of their pathogenic mechanisms (99,100).

S. aureus carries a number of virulence genes that confer strong pathogenic ability and can cause various infectious diseases. Nuc (staphylococcal thermonuclease) encodes a thermostable nuclease essential for immune evasion and biofilm dispersion. Nuc is a key marker for determining the pathogenicity of *S. aureus* (107,108). The Panton-Valentine leukocidin (PVL) gene encodes a cytotoxin that specifically lyses phagocytes (neutrophils and macrophages), depleting frontline immune defenses, disrupting neutrophil extracellular trap formation, and creating favorable conditions for bacterial dissemination (109-111).

In food poisoning, *S. aureus* secretes a large number of enterotoxins, such as those encoded by *sea-see* (encoding the superantigenic staphylococcal enterotoxins A and E, respectively) genes. These enterotoxins are thermostable and cannot be inactivated at conventional cooking temperatures. Following human ingestion of contaminated food, enterotoxins act on the intestinal nervous system, stimulate the vomiting center and cause severe vomiting, diarrhea and other symptoms that endanger health (112,113).

Skin infections are a common type of *S. aureus* infection. The proteins encoded by the fibronectin-binding protein genes (*fnbA* and *fnbB*) help bacteria adhere to the extracellular matrix of skin tissue, and coagulase encoded by the coagulase gene (*coa*) and von Willebrand factor-binding protein encoded by the coagulase-like gene (*cal*) promote plasma coagulation to form a fibrin barrier, resisting phagocytosis by immune cells. *S. aureus* can cause skin and soft tissue infections, such as furuncles, carbuncles and cellulitis, as well as other typical symptoms, such as local redness, swelling, elevated temperature, pain, and suppuration (24,25,106).

M. tuberculosis. *M. tuberculosis*, a pathogen that causes tuberculosis, causes infections worldwide (1,2). Virulence genes serve key roles in latent infections, drug resistance and pathogenesis (Table III) (86). Virulence genes of the *dosR* regulatory system serve an important role in the latent infection stage of *M. tuberculosis*. When the bacterium enters the host and is exposed to stressful environments such as hypoxia and nutrient deprivation, the *dosR* gene is activated, which regulates the expression of a series of downstream genes and causes the bacterium to enter a dormant state (30,33). The *dosR*-regulated genes *Rv2627* (HspX) and *Rv2628* (universal stress protein) maintain bacterial quiescence and resist immune clearance (33,87), reactivating bacteria under defined host immunosuppressive conditions including TNF-α level decrease, CD4+ T cell depletion, or hypoxia alleviation to cause reinfection (30,33,34). For example, in latently infected macrophages, *M. tuberculosis* expressing *dosR*-regulated genes survive for a long time and evade host immune surveillance. Once host immunity decreases, the bacteria recover

Table III. Classification of virulence genes in bacteria.

Authors, year	Bacteria name	Virulence gene	Coding product	Type of infection	Functional role and pathogenic impact	(Refs.)
Ullah *et al*, 2023; Zhang *et al*, 2020	*Escherichia coli*	*fimH*	Type 1 fimbriae tip protein	Urinary tract	Binds mannose receptors on uroepithelialcells; co-localized with *blaCTX-M* on *IncF* plasmids in ESBL strains; expression regulated by gut microbiota SCFAs	(5,88)
Ullah *et al*, 2023		*papC*	P pilus-associated protein	Urinary tract	Mediates bacterial adhesion to urethral epithelium via P-pili assembly; *papC* gene prevalence is significantly higher in ESBL-producing strains compared with non-ESBL-producing strains	(5)
Ullah *et al*, 2023		*hlyA*	α-hemolysin	Bloodstream/ tissue	Forms pores in host cell membranes (erythrocytes/ leukocytes), causing lysis and inflammation; higher prevalence in ESBL strains	(5)
Yang *et al*, 2023; Watanabe *et al*, 2024		*esc* (*T3SS*)	T3SS	Enteric	Forms molecular syringe to inject effectors (such as *Tir*) into intestinal cells, inducing actin pedestal formation and barrier disruption	(27,94)
Watanabe *et al*, 2024		*eae*	Intimin adhesin	Enteric	Mediates tight attachment to epithelial cells, triggering cytoskeletal rearrangement	(94)
Liu *et al*, 2020; Li *et al*, 2024	*Staphylococcus aureus*	*agr* locus	Quorum-sensing regulator	Skin/soft tissue, pneumonia	Orchestrates virulence phase transition via quorum sensing; upregulates *fnbA*-mediated adhesion in early infection, then switches to *hla*/*PVL* toxin expression during proliferation; pharmacologically inhibited by savirin (a small-molecule agr quorum-sensing inhibitor)	(98,101)
Peng *et al*, 2022		*icaABCD*	Exopolysaccharide synthase	Medical device	Synthesizes biofilm matrix; decreases antibiotic penetration and phagocytosis; upregulated by neutrophil ROS	(35)
Karimzadeh *et al*, 2022		*nuc*	Thermostable nuclease	Skin/ bloodstream	Maintains activity at high temperatures; key pathogenicity marker	(107)
Hodille *et al*, 2020		*PVL*	Panton-Valentine leukocidin	Necrotizing pneumonia	Lyses leukocytes; co-integrated with *SCCmec* (*mecA*) in hypervirulent MRSA	(109)
Haghi *et al*, 2021		*sea-see*	Enterotoxin	Food poisoning	Heat-stable; stimulates vomiting center, causing severe diarrhea	(113)
Speziale *et al*, 2020		*fnbA*/*fnbB*	Fibronectin-binding protein	Skin	Mediates adhesion to extracellular matrix of skin tissue	(24)
Claes *et al*, 2017		*coa*/*cal*	Coagulase	Skin	Promotes plasma coagulation to resist phagocytosis	(25)

Table III. Continued.

Authors, year	Bacteria name	Virulence gene	Coding product	Type of infection	Functional role and pathogenic impact	(Refs.)
Cheng *et al*, 2024; Liu *et al*, 2024	*Mycobacterium tuberculosis*	*dosR*	Hypoxia-responsive regulator	Latent/ reactivated TB	Induces dormancy in granuloma; silencing by host IFN-γ leads to reactivation and ESAT-6 release	(30,34)
Pei *et al*, 2024		*rpoB*	RNA polymerase β-subunit	Drug-resistant TB	Mutations confer rifampicin resistance and alter global virulence gene expression	(85)
Gao *et al*, 2024		*pks*	Polyketide synthase	Active TB	Synthesizes wax D for cell wall integrity, resisting lysosomal degradation	(31)
Takahashi-Kanemitsu *et al*, 2023	*Helicobacter pylori*	*cagA*	Cytotoxin-associated protein	Gastric ulcer/ cancer	Injected via T4SS, disrupts cell polarity and promotes precancerous lesions	(117)
Son *et al*, 2025		*vacA*	Vacuolating cytotoxin	Gastric	AB toxin induces mitochondrial apoptosis; s1/m1 subtype more virulent	(118)
Sedaghat *et al*, 2014		*iceA*	Inducible catalase	Gastric colonization	Associated with gastric mucosal colonization and inflammation	(120)
Sedaghat *et al*, 2014		*oipA*	Outer membrane inflammatory protein	Gastric disease	Modulates bacteria-host cell interaction and immune regulation	(120)
Sedaghat *et al*, 2014		*babA*	Blood group antigen-binding adhesin	Gastric colonization	Mediates binding to blood group antigens on gastric epithelium	(120)
Song *et al*, 2024	*Klebsiella pneumoniae*	*iucA* (hvKP)	Aerobactin siderophore	Liver/lung abscesses	Enhances iron acquisition in iron-limited host environments	
Song *et al*, 2024; Jiang *et al*, 2025		*rmpA* (hvKP)	Mucoid phenotype regulator	Invasive	Upregulates capsular polysaccharide for hypermucoviscosity and antiphagocytic activity	(9,13)
Song *et al*, 2024; Jiang *et al*, 2025		*blaKPC* (CRKP)	KPC carbapenemase	Hospital-acquired	Co-localized with *rmpA* on *pLVPK* plasmid in hypervirulent-resistant strains; driven by ICU antibiotic pressure	(9,13)
Zhang *et al*, 2023		*blaCTX-M* (cKP)	ESBL enzyme	Opportunistic	Confers resistance to β-lactam antibiotics in classical strains	(10)

fimH, fimbrial adhesion H gene; *blaCTX-M*, cefotaxime-Munich β-lactamase; IncF, incompatibility group F plasmid; ESBL, extended-spectrum β-lactamase; SCFA, short-chain fatty acids; *papC*, pyelonephritis-associated pilus assembly protein C; *hlyA*, α-hemolysin gene; *esc*, enteropathogenic secretion genes; T3SS, type III secretion system; Tir, translocated intimin receptor; *eae*, *E. coli* attaching and effacing gene; agr, accessory gene regulator; fnb, fibronectin-binding protein; *PVL*, Panton-Valentine leukocidin; *hla*, α-hemolysin; *icaABCD*, intracellular adhesion locus ABCD; SCCmec, staphylococcal cassette chromosome mec; *nuc*, staphylococcal thermonuclease; mecA, methicillin resistance determinant A; MRSA, methicillin-resistant *Staphylococcus aureus*; *sea-see*, staphylococcal enterotoxins A-E; *coa*/*cal*, coagulase/coagulase-like protein; *dosR*, dormancy survival regulator; TB, tuberculosis; *rpoB*, RNA polymerase β-subunit; *pks*, polyketide synthase; *cagA*, polyketide synthase; *vacA*, vacuolating cytotoxin gene A; AB, A subunit (active) + B subunit (binding); *iceA*, inducible catalase; *oipA*, outer membrane inflammatory protein; *babA*, blood group antigen-binding adhesin; *iucA*, aerobactin synthesis gene; hvKP, hypervirulent *K. pneumoniae*; *rmpA*, regulator of mucoid phenotype A; *blaKPC*, *K. pneumoniae* carbapenemase; CRKP, carbapenem-resistant *K. pneumoniae*; cKP, classical *K. pneumoniae*; ICU, intensive care unit; ESAT-6, early secreted antigenic target 6 kDa; pLVPK, plasmid for *K. pneumoniae* virulence; ROS, reactive oxygen species; T4SS, type IV secretion system.

and restart the infection process, leading to tuberculosis recurrence (30,34).

The emergence of drug-resistant *M. tuberculosis* strains poses a challenge for the prevention and control of tuberculosis. There is a complex association between bacterial virulence and drug resistance. Studies have shown that certain drug-resistant gene mutations may indirectly affect the regulation of virulence gene expression (85,114-116), such as the RNA polymerase beta subunit gene (*rpoB*) gene mutation leads to rifampicin resistance. The *rpoB* protein, which is the β-subunit of RNA polymerase, is involved in transcription initiation. Mutation of the *rpoB* protein not only makes bacteria resistant to rifampicin, but may also change the global expression profile of the transcriptome, thereby affecting the transcription levels of virulence genes (85,114-116). In addition, virulence genes associated with cell wall synthesis in *M. tuberculosis*, such as the *pks* gene cluster, which is involved in the synthesis of lipid components in the cell wall, are key for the survival and pathogenesis of this species. These genes confer specialized structural and biophysical properties to the cell wall, notably through the production of phenolic glycolipids (PGLs) that form a hydrophobic barrier blocking lysozyme access to peptidoglycan, phthiocerol dimycocerosates (PDIMs) creating a waxy layer impermeable to cationic antimicrobial peptides like defensins, sulfolipids (SL-1) neutralizing reactive oxygen species and inhibiting phagolysosomal fusion, and mycolic acids constituting a rigid mycomembrane that restricts permeability to bactericidal enzymes (31,32). Collectively, these adaptations enable resistance to host immune effectors and facilitate persistence within macrophages and granulomas (31,32,86). At the same time, they affect the interaction between bacteria and host cells, which is conducive to the colonization and spread of bacteria in host tissue, leading to typical pathological changes in tuberculosis, such as lung tissue damage and cavity formation (31,86). Tuberculosis endangers the function of the respiratory system and the life and health of patients (31,32,86). In addition, *M. tuberculosis* secretes small-molecule effectors during infection to regulate signaling pathways in host cells, inhibit the host immune response and create favorable conditions for bacterial survival and proliferation (30,34,87). This is associated with the regulation of virulence gene expression (30,33,87).

Helicobacter pylori. *H. pylori* is a key pathogen in gastric disease and its virulence gene spectrum is associated with its pathogenicity (Table III) (117,118). *H. pylori* encodes the cytotoxin-associated gene A (*cagA*) that produces the CagA effector protein translocated into gastric epithelial cells via the type IV secretion system (117), and the vacuolating cytotoxin gene A (*vacA*) that encodes the VacA toxin capable of inducing vacuolar degeneration and apoptosis in host cells (118). Specifically, the CagA protein is phosphorylated by intracellular kinases and interacts with Src homology 2 (SH2) domain-containing proteins to activate downstream signals, which leads to cytoskeletal rearrangement, cell proliferation and migration abnormality, loss of cell polarity and cell dispersion in gastric mucosal epithelial cells, thereby aggravating inflammation and promoting the development of precancerous lesions (117). Several studies have shown that patients infected with CagA-positive *H. pylori* strains have a higher risk of gastric cancer and duodenal ulcers than those infected with CagA-negative strains (76,117).

VacA toxin encoded by the *vacA* gene, as an active-binding (AB) toxin composed of a cell-binding B subunit and an enzymatically active A subunit, can cause vacuolization of gastric epithelial cells. The B subunit mediates the binding of the toxin to the cell surface receptor, and the A subunit enters the cell and acts on organelles, such as the mitochondria, to interfere with the intracellular ion balance and induce apoptosis. The cytotoxicity of distinct VacA subtypes exhibits variability. Specifically, s1/m1 strains typically demonstrate higher virulence compared to s2/m2 strains and are more prone to inducing severe gastric lesions (117,118).

In addition to *cagA* and *vacA*, genes such as inducible catalase (*iceA*), outer membrane inflammatory protein (*oipA*) and blood group antigen-binding addin (*babA*) serve key roles in *H. pylori* pathogenesis. The *iceA* gene is associated with bacterial colonization and inflammation of the gastric mucosa. The expression of *oipA* affects the interaction between bacteria and host cells and participates in immune regulation in the host. The *babA* gene helps bacteria adhere to specific blood group antigens on the surface of gastric epithelial cells, enhances colonization and creates conditions for subsequent infection. These virulence genes cooperate with each other so that *H. pylori* can survive and reproduce in the harsh environment of the stomach, gradually inducing gastritis, gastric ulcers and gastric cancer (119,120).

Klebsiella pneumoniae. *K. pneumoniae* is divided into hypervirulent (hv) and classical *K. pneumoniae* (cKP) according to virulence and drug resistance. hvKP, the hypervirulent form of *K. pneumoniae*, usually carries a virulence plasmid that contains siderophore synthesis genes, including the aerobactin synthesis gene *iucA* encoding lysine N6-hydroxylase, together with key virulence genes such as the regulator of mucoid phenotype A (*rmpA*). The *iucA* gene encodes proteins associated with siderophore synthesis, which help bacteria compete for iron to meet growth and reproduction needs in a host environment where iron uptake is limited. The *rmpA* gene enhances the anti-phagocytic ability and pathogenicity of bacteria by regulating the synthesis of bacterial polysaccharides and capsule formation, which confers a hypermucoviscous phenotype that facilitates colonization and spread in the host (9,121-123).

By contrast, cKP, an opportunistic pathogen, is relatively weak in terms of virulence but has a strong ability to acquire drug-resistant genes. It typically integrates drug resistance genes through HGT (such as plasmid conjugative transfer and transposon skipping) to form multidrug-resistant phenotypes. Common resistance genes include ESBL genes (such as β-lactamase Temoniera), *blaSHV* (beta-lactamase sulfhydryl variable) and β-lactamase cefotaxime-Munich), carbapenemase genes [such as *KPC* (*K. pneumoniae* carbapenemase), *VIM* (Verona integron-encoded metallo-β-lactamase) and *NDM* (New Delhi metallo-β-lactamase)] and quinolode resistance genes [such as *qnrA* (quinolone resistance A), *qnrB* (quinolone resistance B) and *qnrS* (quinolone resistance S)]. Consequently, it is resistant to numerous antibiotics, such as β-lactams, carbapenems and fluoroquinolones, which poses challenges to clinical treatment (124-126).

In recent years, *K. pneumoniae* strains with high virulence and multi-drug resistance characteristics have emerged (9,13,124,127) (Table III). These strains not only infect healthy individuals, causing liver and lung abscesses, endophthalmitis and other invasive infections, but also limit the choice of treatment drugs due to drug resistance, prolong the course of the disease and increase mortality rates. In mainland China, Southeast Asia, and healthcare-constrained settings, multidrug-resistant strains account for a notable proportion of hospital-acquired *K. pneumoniae* infection, among which the 30-day mortality rate of infection caused by hypervirulent and drug-resistant strains is notably higher than that of non-drug-resistant strains, highlighting the need for enhanced prevention and control of *K. pneumoniae* infections (9,128).

Viral pathogens

Novel coronavirus. SARS-CoV-2 is the main causative agent of the coronavirus disease (COVID-19) pandemic. Its virulence genes serve a key role in the transmission and pathogenesis of the virus and have become the focus of research worldwide (129). The *S* gp gene of SARS-CoV-2 encodes S gp, a trimeric structure on the viral surface that mediates host cell entry. The S protein binds the ACE2 receptor, a membrane protein highly expressed in lung alveolar epithelial cells, heart and intestines, via its RBD. This interaction triggers membrane fusion, enabling the viral RNA genome to enter host cells and initiate replication (19,130). The role of ACE2 in regulating blood pressure and inflammation explains why SARS-CoV-2 infection typically leads to extrapulmonary complications, including acute respiratory distress syndrome and cardiovascular injury (131). The S protein gene frequently mutates. Certain variants, such as Δ and o, have amino acid mutations in the RBD region that enhance the affinity of the virus for the ACE2 receptor, facilitating viral entry into cells and improving the infectivity of the virus (132). A study on variants showed that the RBD mutation of the S protein in the o variant increases the binding affinity of o to the ACE2 receptor compared with the original strain, which was closely related to the rapid spread of this variant worldwide and the peak of infection (39,133).

Other accessory protein genes of SARS-CoV-2 also serve important roles in pathogenesis. For example, the protein encoded by the Open Reading Frame 3a (*ORF3a*) gene induces an inflammatory response in host cells, disrupts the intracellular ion balance and triggers apoptosis, leading to tissue damage and aggravation of the disease. Studies have found that *ORF3a* gene expression is increased in patients with severe COVID-19, and associated with the occurrence of inflammatory factor storm, indicating that it serves a role in the progression of the disease (134,135) (Table IV).

Influenza viruses. The influenza virus, a seasonal circulating RNA virus, frequently causes global influenza epidemics, posing a threat to human health. Variations in virulence genes are associated with antigenic drift and host adaptation of influenza viruses, which are key factors in the continuous evolution of viruses and changes in pathogenicity (Table IV). Influenza viruses are divided into three types according to the antigenicity of the nucleoprotein and matrix protein components. Among these, the influenza A virus is the most pathogenic, prone to mutation and the main pathogen responsible for influenza pandemics (53,136).

The *HA* and neuraminidase (*NA*) genes of the influenza A virus are key virulence genes. The HA protein recognizes and binds to host cell surface receptors, mediating virus invasion. Mutations in this gene change in the antigenicity of the HA protein, causing antigenic drift. For example, there are certain point mutations in the *HA* gene of seasonal influenza virus strains circulating each year, which allow the virus to evade the immune response induced by previous infection or vaccination, leading to recurrent outbreaks (37,46,53). Studies have shown that mutations in key antigenic sites of the HA protein of influenza A (H3N2) viruses decrease the protective efficacy of influenza vaccines and increase the infection rate (37,46).

Neuraminidase, encoded by the *NA* gene, serves a key role in viral release. By cleaving sialic acid residues, progeny virions can be separated from the surface of infected cells and spread. Variations in the *NA* gene also affect the pathogenicity and transmission of viruses. Some highly pathogenic avian influenza viruses, such as *H5N1* and *H7N9*, have a mutation in the *NA* gene that not only enhances the viral reproduction and transmission efficiency in poultry but also confers the ability to cross the species barrier to infect humans, causing a public health crisis (137-139).

In addition to the *HA* and *NA* genes, the influenza A virus polymerase genes *PB1*, *PB2*, and *PA* encode the three subunits of the RNA-dependent RNA polymerase complex. *PB1* functions as the core RNA polymerase *PB2* supplies capped RNA primers and *PA* provides endonuclease activity for cap snatching together ensuring efficient viral RNA synthesis and replication. The polymerase complex encoded by these genes is responsible for the transcription and replication of the viral genome, and its variation can affect the adaptation of the virus to different host cells. For example, certain mutations in the *PB2* gene enable influenza viruses to better adapt to the intracellular environment of human cells, enhance the replication efficiency of the virus in the body and aggravate infection. During the 2009 influenza A (H1N1) pandemic, specific mutations in the *PB2* gene of epidemic strains were associated with the high pathogenicity of the virus and its rapid spread in the population (140,141).

HIV. HIV is the causative agent of acquired immune deficiency syndrome (AIDS), and its virulence genes serve a central role in immune evasion and disease progression (Table IV). HIV is a retroviral RNA virus that primarily attacks $CD4^+$ T lymphocytes in the human immune system and gradually destroys the human immune defense, making patients vulnerable to a variety of opportunistic infections and tumor invasion (45,142).

Envelope gp120 and gp41, encoded by the HIV *env* gene, are key virulence factors. gp120 is responsible for recognizing and binding CD4 receptors and co-receptors (such as C-C motif chemokine receptor 5 and C-X-C motif chemokine receptor 4) on the surface of host immune cells (such as $CD4^+$ T lymphocytes), mediating the fusion of the viral envelope with the host cell membrane, enabling entry of the viral core into the cell, and initiating the infection process (38,41,42). During infection, the *env* gene frequently mutates, particularly in the V3 loop region of the gp120 protein (52). The high variability in the amino acid sequence allows the virus to evade recognition by neutralizing antibodies produced by the immune system and achieve immune escape (41,42,52). Studies have

Table IV. Classification of virulence genes in viral pathogens.

Authors, year	Viral pathogen	Virulence gene	Coding product	Type of infection	Pathogenic mechanism	(Refs.)
Addetia *et al*, 2023; Xu *et al,* 2022; Suzuki *et al*, 2022; Uriu *et al*, 2023	SARS-CoV-2	Spike protein	Trimeric spike glycoprotein	COVID-19	Mediates viral entry by binding ACE2 receptors through receptor-binding domain; key mutations (such as *N501Y* in o variants) enhance ACE2 binding affinity; ACE2 dysregulation contributes to extrapulmonary complications, including acute respiratory distress syndrome; continuous antigenic drift enables pandemic persistence	(19,130-132)
Su *et al*, 2023; Miao *et al*, 2021		*ORF3a*	ORF3a ion channel protein		Induces NLRP3 inflammasome activation, disrupts intracellular ion homeostasis and triggers apoptosis; upregulated in patients with severe COVID-19, associated with cytokine storm severity and thrombotic complications	(134,135)
Chauhan *et al*, 2022; Luo *et al*, 2024; Ding *et al*, 2024	Influenza A virus	*HA*	HA	Influenza	Facilitates viral attachment to sialic acid receptors; antigenic drift (such as mutations in *H3N2* HA1 domain) alters antibody epitopes, enabling immune evasion and decreasing vaccine efficacy; directly contributes to endothelial damage in severe pneumonia	(37,46, 53)
Lederhofer *et al*, 2024; Wang *et al*, 2024; Liao *et al*, 2017		Neuraminidase	Neuraminidase		Cleaves sialic acid residues to release progeny virions; mutations in avian strains (such as *H5N1* and *H7N9*) enhance enzymatic efficiency and zoonotic adaptation	(137-139)
Zhang *et al*, 2024; Günl *et al*, 2023		Polymerase genes *(PB1*, *PB2* and *PA)*	RNA-dependent RNA polymerase complex		Governs viral genome replication; *PB2* mutations (such as E627K) enhance polymerase activity in mammalian cells; key for pathogenicity in pandemic strains (such as 2009 *H1N1*)	(140,141)
Zhang *et al*, 2023; Xie *et al*, 2024; Fu *et al*, 2024; Bennett *et al*, 2021 Qian *et al*, 2023	HIV	*env*	Envelope gp120/gp41	AIDS	gp120 binds $CD4^+$/$CCR5^+$ T cells via V3 loop; rapid glycosylation changes in hypervariable regions enable immune evasion; primary driver of cell-to-cell transmission	(41-45)
Emert-Sedlak *et al*, 2024; Li *et al*, 2024		*tat*	Tat		Enhances viral transcription via TAR RNA binding; induces bystander cell apoptosis and $CD8^+$ T cell exhaustion	(38,52)

Table IV. Continued.

Authors, year	Viral pathogen	Virulence gene	Coding product	Type of infection	Pathogenic mechanism	(Refs.)
Fu *et al*, 2024		*rev*	Rev		Enables nuclear export of unspliced viral RNA; maintains latent reservoirs by suppressing immunogenic protein expression	(43)
Emert-Sedlak *et al*, 2024; Li *et al*, 2024; Biradar *et al*, 2024; Schenck *et al*, 2024		*nef*	Nef		Downregulates MHC-I and CD4 via clathrin-mediated endocytosis; enhances virion infectivity and promotes immune evasion	(38,52,143, 144)

SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; COVID, Coronavirus disease 2019; ACE2, angiotensin-converting enzyme 2; *ORF3a*, Open Reading Frame 3a; *HA*, hemagglutinin; PB, polymerase basic; PA, polymerase acidic; HIV, human immunodeficiency virus; *env*, envelope gene; gp, glycoprotein; AIDS, acquired immune deficiency syndrome; *tat*, transactivator of transcription; TAR, Trans-activation response element; *rev*, regulator of expression of virion proteins; *nef*, negative regulatory factor; MHC-I, major histocompatibility complex class I; CCR, C-C chemokine receptor; NLRP3, NACHT, LRR and PYD domains-containing protein 3.

shown that in HIV-infected individuals, *env* gene mutations gradually accumulate as the disease progresses (38,41,42,52). The neutralizing antibodies generated against early HIV strains are no longer able to effectively neutralize later variants, resulting in the continued replication of the virus and continued impairment of the immune system (41,42).

HIV regulatory genes, such as transactivator of transcription (*tat*) and regulator of expression of virion proteins (*rev*), serve pivotal roles in temporal regulation of viral gene expression. The Tat protein, encoded by the *tat* gene, enhances viral transcription, promotes efficient viral gene expression and ensures viral replication within host cells (38). The Rev protein, encoded by the *rev* gene, regulates the splicing and transport of viral mRNA, ensuring the production of complete viral genomic RNA and structural proteins, thereby maintaining persistent infection and pathogenicity (43). Aberrant expression of these regulatory genes is associated with the progression of HIV infection. For example, in patients with AIDS, elevated expression of the *tat* gene is frequently observed, leading to increased viral replication in immune cells, accelerated depletion of $CD4^+$ T lymphocytes and a compromised immune system (52).

In addition, the negative regulatory factor (Nef) protein encoded by the HIV *nef* gene has a variety of functions that interfere with the signal transduction pathways of host cells, downregulate the expression of CD4 receptors and major histocompatibility complex class I molecules on the cell surface, and reduce the binding opportunity of neutralizing antibodies when the virus infects the cells. This decreases the probability of infected cells being recognized and killed by cytotoxic T lymphocytes, facilitating viral immune escape and persistent infection (38,52). Virulence of HIV strains with *nef* deletion was weakened in in BLTS-humanized mice challenged with HIV-1 and in C57BL/6 mice infected intracranially with EcoHIV-ΔNef, indicating that the Nef protein serves a key role in the pathogenesis of HIV (38,52,143,144).

Emerging viral pathogens and virulence evolution

Zika virus (ZIKV). ZIKV is a significant global health threat because of its association with congenital microcephaly and Guillain-Barré syndrome (145). Non-structural protein 5 (NS5) protein, encoded by the *NS5* gene, is a key virulence factor (145). *NS5* mediates immune evasion by degrading host STAT2, a key component of the type I IFN signaling pathway (145). Structural studies have revealed that *NS5* binds to the STAT2 SH2 domain, triggering its ubiquitination and proteasomal degradation (145-147). This suppression of IFN responses allows ZIKV to cross the placental barrier, as demonstrated in murine models, where *NS5*-mutant strains (such as *R99K*) showed decreased fetal infection rates (146,147). Recent outbreaks in Southeast Asia have highlighted *NS5* mutations that enhance viral persistence in placental tissue, underscoring the need for targeted antiviral strategies (147,148).

Ebola virus. The Ebola virus gp, encoded by the *GP* gene, is key to hemorrhagic pathogenesis. gp facilitates viral entry by binding host T-cell immunoglobulin and mucin domain-1 and Niemann-Pick C1 (NPC1) receptors on endothelial cells, inducing vascular leakage and systemic inflammation (149). A single amino acid mutation (T544I) in the *GP* receptor-binding domain increases viral entry efficiency, as shown in pseudovirus

assays (150). This mutation was associated with higher case fatality rates in recent outbreaks in the Democratic Republic of the Congo (150). Additionally, the mucin-like domain of *GP* masks viral epitopes, evading neutralizing antibodies and complicating vaccine design (150).

Nipah virus (NiV). NiV, a paramyxovirus with high zoonotic potential, uses its fusion (F) protein (encoded by the *F* gene) to mediate cell-cell fusion and neuronal spread (151). Cryo-electron microscopy studies have demonstrated that the *F* protein undergoes conformational changes to expose a hydrophobic fusion peptide, enabling syncytia formation in the respiratory epithelium and in neurons (151,152). The E447K mutation in the receptor-binding domain of the F protein enhances the affinity for ephrin-B2/B3 receptors in human hosts, thereby increasing the spillover risk from bat reservoirs (151). Outbreaks in Malaysia and Bangladesh have highlighted the capacity of *NiV* for rapid adaptation, necessitating surveillance of *F* gene evolution in animal reservoirs.

Fungal pathogens

C. albicans. As a common opportunistic pathogenic fungus, *C. albicans* can easily cause superficial and deep infections in patients with low immune function or microecological imbalances. The regulation of virulence gene expression is associated with morphological transformation, host immune escape and drug resistance, which are key entry points for studying the pathogenic mechanisms of fungi (Table V) (18,153).

C. albicans is characterized by a unique biphasic transition between the yeast and hyphal phases, which is regulated by various virulence factors. For example, EFG1 gene is a key transcription factor in the regulation of morphological transition. The encoded protein binds the promoter region of hyphal-specific genes, activates the expression of hyphal-associated genes and promotes the transition from the yeast phase to the hyphal phase. When the host environment changes, such as the temperature rising to 37˚C, nutrient composition changes or serum presence, the expression of the EFG1 gene is upregulated, which triggers the morphological transformation of *C. albicans* (16,18,54,55,67). Hyphae penetrate the epithelial cell layer and invade deep tissue, resulting in the spread of infection (16,54).

The virulence genes of *C. albicans* also serve a key role in immune escape. The products encoded by Sap family genes secreted by *C. albicans* degrade immune molecules, such as immunoglobulin and complement components, on the surface of host cells, thereby destroying the host immune defense system (154). Studies have found that the expression of the Sap gene is upregulated during *C. albicans* infection (154-156). Secreted proteases hydrolyze host antimicrobial peptides, decrease local immune activity and create favorable conditions for fungal survival (55,157). In addition, *C. albicans* modifies the components of the cell wall, including increasing the phosphorylation of mannan or changing the synthesis of chitin, decreasing the immunogenicity of the cell wall, and avoiding recognition by the host immune system to achieve immune escape (56).

Drug resistance in *C. albicans* is a problem worldwide. There are complex interactions between virulence and drug resistance genes. Several studies have shown that virulence genes involved in biofilm formation, such as *Bcr1* and Cyanobacterial phytochrome 1, are associated with drug resistance (63,158). As a protective structure formed by *C. albicans* at the site of infection, biofilms not only block the penetration of antifungal drugs but also change the drug metabolism pathway in fungal cells, leading to drug resistance. When the *Bcr1* gene is activated, it upregulates the expression of biofilm-associated genes, promotes the synthesis of extracellular polysaccharides, enhances the stability of biofilms and improves the tolerance of fungal cells to antifungal drugs (18). In-depth exploration of the mechanism of *C. albicans* virulence genes in morphological transformation, immune escape and drug resistance is expected to provide key targets for the development of novel antifungal drugs and treatment strategies that effectively address the challenges posed by *C. albicans* infection.

Aspergillus spp. Aspergillus is a filamentous fungus widely distributed in nature. *A. fumigatus* is one of the most common pathogenic species and causes invasive aspergillosis, especially in immunocompromised patients, with a high mortality rate. For example, in Quebec, Canada, from March 2018 to December 2018, over 500 cases of invasive aspergillosis were reported annually at McGill University Health Centre, with an overall mortality rate of approximately 25%. However, in cases involving azole-resistant *A. fumigatus* (ARAF), the mortality rate exceeded 60%. Globally, it is estimated that there are at least 300,000 cases of invasive pulmonary aspergillosis (IPA) annually, with mortality rates ranging from 44% to 80%. In China, a systematic review reported that *A. fumigatus* accounted for 75.14% of all *Aspergillus* isolates, highlighting its prevalence as a pathogen. The virulence of *Aspergillus* species is affected by various environmental factors, such as temperature, pH, humidity, nutrient availability, oxygen levels, and the presence of organic matter. The pathogenic mechanism of invasive *Aspergillus* infection involves multiple factors, such as interaction with host cells, immune escape and toxin secretion (159).

Environmental factors serve a key role in the regulation of *Aspergillus* virulence gene expression. When the ambient temperature is close to the human body temperature (37˚C), the expression of several virulence genes in *A. fumigatus* changes. For example, the expression levels of the chitin synthase gene, which is involved in cell wall synthesis, are upregulated, promoting cell wall thickening and enhancing the resistance of the fungus to host immune responses. Conidium germination and mycelial growth-associated genes, such as bristle A and abacus A (*abaA*), are also activated, accelerating fungal colonization and diffusion in the host (159). In addition, changes in nutrient composition affect virulence gene expression. The expression of certain virulence genes, such as the hexokinase gene involved in glucose metabolism and the catalase (*cat*) gene regulating the oxidative stress response, changes to adapt to nutrient deficiency and oxidative stress to ensure the survival and proliferation of *A. fumigatus* under low-glucose and hypoxic environments in the host (73,160).

Invasive infection by *Aspergillus* involves a pathogenic link between the fungus and host cells. Adhesion molecules, such as the hydrophobic protein *RodA*, recognize and bind receptors on the surface of host epithelial and endothelial cells to mediate fungal adhesion, which is the initial step of host infection (61). Once adhesion is successful, *Aspergillus* hyphae destroy host cell barriers and invade tissues through

Table V. Classification of virulence genes in fungal pathogens.

Author/s, year	Fungal pathogen	Virulence gene	Coding product	Type of infection	Pathogenic mechanism	(Refs.)
Glazier, 2022; Liang *et al*, 2024	*Candida albicans*	EFG1	Transcription factor	Superficial/deep infection in immunocompromised hosts	Master regulator of yeast-to-hyphal transition at body temperature; binds promoters of hyphal-specific genes (*HWP1* and *ALS3*); hyphae penetrate epithelial barriers and secrete *SAP*; in response to macrophage-derived ROS), EFG1 activates hyphal morphogenesis coupled with *Sap2-6* secretion to degrade antimicrobial peptides	(16,54)
Lin *et al*, 2023; Naglik *et al*, 2003		*Sap2-Sap6*	SAP	Mucosal and systemic	Degrade host immunoglobulin, complement components and antimicrobial peptides; upregulated during mucosal invasion; *SAP5* cleaves E-cadherin, disrupting epithelial barriers	(55,154)
Nobile *et al*, 2006; Nobile and Mitchell, 2005		*Bcr1*	Biofilm regulator	Catheter-associated	Upregulates exopolysaccharide synthesis genes (*ALS1* and *ALS3*) for biofilm matrix formation; biofilms decrease antifungal penetration efficiency and are associated with persistent infection; cooperates with *Cph1* in adhesion maintenance	(63,158)
Liang *et al*, 2024; Zhang *et al*, 2024		*HWP1*	Hyphal wall protein	Mucosal colonization	Mediates adhesion to epithelial cells via covalent cross-linking to host transglutaminase; essential for tissue penetration and resistance to mechanical clearance	(54,57)
Latgé and Chamilos, 2019; Fréalle *et al*, 2013	*Aspergillus fumigatus*	*chs* (*chsA-G*)	Chitin synthase	Invasive aspergillosis	Upregulated at human body temperature for cell wall thickening; chitin layers resist lysosomal degradation in macrophages; mutants show decreased virulence in infection models	(159,160)
Liu *et al*, 2023; Luptáková *et al*, 2023		*SidA*	Siderophore synthase	Pulmonary	Synthesizes fusarinine C for iron acquisition; hypoxia-inducible via host HIF-1α signaling; critical for growth in iron-limited lung environments	(70,71)
Carrion Sde, 2013		*RodA*	Hydrophobin	Respiratory adhesion	Coats conidia to mediate adhesion to pulmonary epithelial cells; masks β-glucan to decrease immune recognition; deletion increases phagocytosis efficiency	(61)

EFG, enhanced filamentous growth protein; HWP, hyphal wall protein; ALS, agglutinin-like sequence; SAP, secreted aspartyl protease; BCR, biofilm and cell wall regulator; CPH, Cyanobacterial phytochrome; chs, chitin synthase; *SidA*, siderophore biosynthesis enzyme A; HIF, hypoxia-inducible factor; *RodA*, rodlet protein A; ROS, reactive oxygen species.

mechanical stress and the secretion of hydrolases, such as proteases and phospholipids (70). The invasive ability of *Aspergillus* mutants lacking hydrolase genes is reduced in murine models of infection, indicating that these hydrolases serve a key role in the pathogenic process (61,161).

Aspergillus also possesses the ability to escape and evade clearance by the host immune system. Melanin in the cell wall of *Aspergillus* has an immunosuppressive effect, which can shield fungal cell surface antigens, decrease the recognition of host immune cells and resist the attack of ROS produced by macrophages. In addition, some secondary metabolites secreted by *Aspergillus*, such as gliotoxin, inhibit the activation, proliferation and function of host immune cells, induce immune cell apoptosis, further weaken host immune defense and create favorable conditions for persistent fungal infection (79). An in-depth study of the association between *Aspergillus* virulence genes and environmental factors, as well as its pathogenic mechanism, is key for the development of precise anti-*Aspergillus* treatment strategies and decreasing the mortality associated with invasive *Aspergillus* (Table V).

C. auris. *C. auris* is a fungal pathogen that uniquely combines multidrug resistance and enhanced virulence (77). Its rapid global spread and persistence in healthcare settings underscore the need to elucidate the molecular interplay between its resistance and pathogenicity (78). A hallmark of *C. auris* is the *ERG11* gene, which encodes lanosterol 14α-demethylase, a target of azole antifungals (62). Mutations in *ERG11*, such as Y132F and K143R, alter the topology of the enzyme active site, thereby reducing azole binding affinity (62). Molecular dynamics simulations have revealed that the Y132F substitution introduces steric hindrance, displacing the triazole ring and increasing binding free energy (62). Clinically, these mutations are associated with elevated minimum inhibitory concentration values and high treatment failure rates (62). Notably, *ERG11*-mutant strains also exhibit thickened biofilms, further impeding drug penetration (74).

The evolution of resistance extends beyond that against azole drugs. Mutations in β-1,3-glucan synthase gene (*FKS1*) drive echinocandin resistance by distorting the catalytic pocket of the enzyme (162). The S639F substitution elevates the IC_{50} of caspofungin (163). *FKS1* mutations are mechanistically linked to enhanced virulence in *C. neoformans*. RNA-sequencing analysis of clinical isolates has demonstrated that *FKS1*-mutant strains upregulate *SAP5*, an aspartic protease, via the activation of the High-Osmolarity Glycerol Mitogen-Activated Protein Kinase (HOG-MAPK) stress-response pathway. This co-regulation translates to a notable increase in epithelial invasion efficiency *in vitro*, as shown by Transwell assays (62). This interaction between resistance and virulence underscores the adaptability of *C. auris* under therapeutic pressure (78).

Environmental persistence is a key aspect of *C. auris* pathogenicity, which is mediated by the *ssa1* gene. Hsp70 enables thermotolerance and biofilm stability (74). Ssa1 expression increases at 42˚C, allowing survival on surfaces for >30 days. This thermotolerance is likely due to the activation of heat-shock proteins and other stress-response mechanisms in C. auris (162). Functional studies of *ssa1*-knockout strains have revealed a substantial reduction in biofilm β-1,3-glucan content and a marked increase in susceptibility to hydrogen peroxide disinfectant (62,74). Furthermore, *ssa1* stabilizes drug efflux pumps (such as *CDR1*), amplifying fluconazole resistance by enhancing efflux capacity (62).

Genomic studies have highlighted the role of mobile genetic elements in the dissemination of resistance-virulence modules (164,165). In the predominant Clade I lineage, *ERG11* and the hyphal adhesin gene *ALS3* co-localize on conjugative plasmids with efficient HGT (166). Murine infection models have demonstrated that plasmid-bearing strains exhibit higher mortality and resistance to combination therapies (such as fluconazole combined with caspofungin) (163,164). This genetic architecture suggests that traditional antifungal strategies, which focus solely on resistance mechanisms, may inadvertently select hypervirulent clones (164).

The *HSP90*-calcineurin axis bridges stress adaptation and immune evasion. Under neutrophil-derived ROS stress, *HSP90* stabilizes calcineurin, promoting hyphal morphogenesis and escape from phagolysosomal killing (167). Pharmacological inhibition of *HSP90* with geldanamycin restores fluconazole susceptibility in resistant strains (168). *C. auris* employs this pathway more aggressively than *C. albicans*, with higher *HSP90* expression in host niches, enabling persistence in immunocompromised patients (62).

4. Application of pathogen virulence gene research in antimicrobial resistance prevention and control

Mechanisms of association between virulence genes and antimicrobial resistance

HGT mediates drug resistance. HGT is a key driver of drug resistance and virulence enhancement in pathogens. Mobile elements, such as plasmids and transposons, spread resistance and virulence genes (10,11). For example, *S. aureus Tn554* transposons carry both erythromycin resistance and toxin genes, increasing the transfer efficiency compared with single-gene elements (12). This demonstrates that transposon-mediated resistance-virulence coupling is not a passive process but is actively driven by transposase-mediated DNA recombination (11).

As circular double-stranded DNA molecules independent of bacterial chromosomes, plasmids autonomously replicate and carry a variety of drug resistance (such as β-lactamase genes that confer bacterial resistance to β-lactam antibiotics) and virulence genes (such as enterotoxin genes of *E. coli* that enhance pathogenicity) (11). Transposons, which are DNA fragments that can jump in the genome, also carry drug resistance and virulence genes. Transposons are randomly inserted into bacterial chromosomes or plasmids, destroying the original gene function or activating silent genes, thereby causing notable changes in bacterial phenotypes. The *Tn554* transposon in *S. aureus* is a mobile genetic element that co-transfers *erm* and *etb*. Erm encodes a methyltransferase that modifies bacterial ribosomes and confers resistance to macrolide antibiotics (such as erythromycin) (75). Etb produces a toxin that cleaves desmoglein-1, a key protein for epidermal cell adhesion, causing staphylococcal scalded skin syndrome (12). This dual gene cargo promotes the spread of multidrug-resistant hypervirulent strains, complicating the clinical management of infection (6,12).

HGT not only accelerates the generation and spread of drug-resistant bacteria but also complicates the virulence spectrum of pathogens, posing numerous obstacles to clinical anti-infection treatment (Table VI).

Biofilm formation and drug resistance regulation. A biofilm is a group survival strategy fortress built by bacteria to cope with harsh environments, particularly in response to antibiotic stress. Biofilm structures are complex, and composed of bacterial cells, extracellular polysaccharides (EPS), proteins, nucleic acids and other components interwoven to form a three-dimensional network structure (35,169).

For example, *P. aeruginosa* is a common clinically refractory infectious pathogen, and its biofilm formation is regulated by various virulence genes such as Pellicle (*Pel*) and Pseudomonas surface polysaccharide I (*PsI*) (Table VI). *Pel* genes encode products involved in exopolysaccharide synthesis and biofilm skeleton construction. The *PsI* gene directs protein secretion, promotes bacterial cell adhesion and aggregation, and stabilizes the biofilm structure. Following biofilm formation, the permeability barrier effect of the biofilm prevents antibiotics from reaching bacteria. Inside the bacteria, the drug concentration is decreased, greatly weakening the antibiotic effect of the drug. Simultaneously, bacteria within biofilms exhibit a sluggish metabolism, reshaping of their gene expression profiles, and activation of resistance genes. These changes further enhance antibiotic resistance and contribute to the formation of persistent, localized infections. This often leads to chronic, recurrent infections in the respiratory and urinary tracts, which are difficult to treat (36,170).

Co-selection of virulence genes and drug resistance

Virulence-resistance colocalization in carbapenem-resistant K. pneumoniae (*CRKP*). The co-selection of virulence and resistance genes has become increasingly common in multidrug-resistant pathogens (127). For example, hvKP strains of CRKP carry the pLVPK plasmid, which integrates both the *rmpA* (regulating mucoid phenotype to enhance antiphagocytic ability) and the beta-lactamase gene encoding Klebsiella pneumoniae carbapenemase gene (encoding carbapenemase) (9,171). The colocalization of the *blaKPC* gene and the *pLVPK*-like virulence plasmid in carbapenem-resistant *K. pneumoniae* (CR-KP) strains has been shown to increase mortality rates in patients with bloodstream infections (13). Specifically, a study conducted between December 2017 and April 2018 in China reported that out of 24 CR-KP isolates recovered from patients with bacteremia, the mortality rate was 66.7%. This highlights the significant impact of the colocalization of resistance and virulence genes on patient outcomes (172). A multicenter study in China showed a strong positive association between the intensity of carbapenem antibiotic use in intensive care units and the spread of the pLVPK plasmid, indicating that antibiotic pressure directly drives 'virulence-resistance' co-evolution in *K. pneumoniae* (127).

Virulence-resistance synergy module in methicillin-resistant S. aureus (MRSA). The Staphylococcal Cassette Chromosome mec (SCCmec) element, carrying the methicillin resistance gene methicillin resistance determinant A (*mecA*) in MRSA often co-integrates with the *PVL* gene (encoding leukocidin) on the chromosome (173). *PVL* toxins weaken innate immunity by disrupting neutrophil extracellular traps, whereas *mecA* confers β-lactam resistance via the variant penicillin-binding protein 2a (174). Data from the European Centre for Disease Prevention and Control showed a notable increase in necrotizing pneumonia cases caused by community-acquired MRSA carrying both PVL and SCCmec IV in 2023, with a higher vancomycin treatment failure rate than that of non-PVL strains (111).

Stress pathway-driven co-evolution in Enterococci. In *Enterococcus faecalis*, the vancomycin resistance gene vancomycin resistance gene A (*vanA*) and the virulence gene gelatinase gene (*gelE*) are co-regulated by the stress factor RNA polymerase sigma factor S (RpoS). Under vancomycin pressure, RpoS activates both the resistance phenotype of *vanA* and the colonization function of *gelE* (which degrades the extracellular matrix). This dual activation increases the intestinal colonization rate of vancomycin-resistant strains in patients with inflammatory bowel disease and enables *Enterococci* to occupy niches in immunocompromised hosts more effectively (91).

Resistance prevention and control strategies based on virulence genes

Targeted drug development. The research and development of drugs targeting virulence factors have provided novel methods to overcome the problem of pathogen resistance in aquaculture. For example, the *agr* quorum-sensing inhibitor savirin selectively suppresses *hla* expression in *S. aureus*, decreasing necrotic skin lesions in murine models without affecting bacterial viability (14,175). This approach minimizes the selective pressure for resistance compared with that of traditional bactericidal antibiotics. Artificial intelligence (AI)-driven quantum mechanics/molecular dynamics (QM/MD) simulations have been used to optimize the design of a peptide inhibitor targeting *S. pneumoniae* adhesin pneumococcal surface protein C, which decreases bacterial colonization in human nasopharyngeal organoids (57). Unlike traditional antibiotics, which act through broad, multi-target mechanisms such as inhibiting cell-wall synthesis, protein translation, or DNA replication, these drugs selectively disrupt specific pathogen-host interactions without globally affecting bacterial viability. By specifically inhibiting their function, the pathogenicity of pathogens is weakened and the harm of infection is decreased, while avoiding the emergence and spread of antibiotic-resistant bacteria (176,177).

A number of studies have focused on developing small-molecule compounds or biological agents that neutralize the activity of bacterial toxins (72,178,179). The primary pathogenic factors of severe diarrhea caused by *Clostridium difficile* are toxins A (*TcdA*) and B (*TcdB*). *TcdA* is an enterotoxin that binds to intestinal epithelial cells, causing cytoskeletal disruption, cell rounding, and detachment, leading to severe inflammation and tissue damage (178,180). *TcdB* is a cytotoxin that is more potent than TcdA, binding to cell receptors and inducing apoptosis, affecting a broader range of cell types and contributing significantly to the cytotoxic effects observed in infections (178,180). Researchers have used structural biology to analyze the three-dimensional structure of *C. difficile*

Table VI. Mechanistic association between virulence genes and drug resistance.

Authors, year	Strategy type	Study type	Pathogen	Genetic or molecular target	Key findings	(Refs.)
Ullah *et al*, 2023	Horizontal gene transfer	Clinical isolate-based study of hospital-acquired infections	*Escherichia coli* and *Klebsiella pneumoniae*	*IncF* plasmid carrying carbapenemase resistance, adhesin (*fimH*) and iron uptake system gene	Plasmid-mediated horizontal transfer contributes to coexistence of drug resistance and virulence, complicating treatment	(5)
Ji *et al*, 2022		Transposon-mediated resistance-virulence co-transfer study	*Staphylococcus aureus*	*Tn554* transposon with erythromycin resistance (*erm*) and exfoliative toxin gene (*etb*)	Transposon movement leads to spread of resistance and enhanced virulence, increasing the difficulty of treating infection	(75)
Zhang *et al*, 2023	Biofilm formation	Biofilm-associated chronic lung infection model	*Pseudomonas aeruginosa*	*Pel* and *Psl* genes regulating biofilm formation	Activation of these genes results in biofilm formation, which decreases drug penetration and enhances drug resistance, causing chronic infection	(72)
Goldsmith *et al*, 2023	Targeted drug development	Structure-based anti-toxin drug development	*Clostridium difficile*	Toxins A (*TcdA*) and B (*TcdB*)	Structural analysis led to the design of a small molecule inhibitor that blocks toxin binding	(178)
Mangal *et al*, 2023		Anti-adhesin peptide development for colonization prevention	*Streptococcus pneumoniae*	Adhesin	Peptide drugs competitively bind adhesin key sites	(179)
Gao *et al*, 2024	Combination therapy	Combination therapy with anti-virulence peptides	*Helicobacter pylori*	Amoxicillin combined with anti-virulence peptide	Combination therapy increases eradication rate compared with single antibiotic use	(183)
Zhang *et al*, 2023		Enzyme-antibiotic combination therapy for biofilm infection	*Pseudomonas aeruginosa*	Tobramycin and specific biological enzyme preparation	Combination therapy enhances bacterial clearance rate	(72)

IncF, incompatibility group F plasmid; *fimH*, fimbrial adhesion H gene; Tn554, transposon 554; erm, erythromycin resistance methylase; etb, erythromycin resistance methylase; Pel, Pel polysaccharide synthesis locus; Psl, Pseudomonas surface polysaccharide locus; Tcd, toxin of *Clostridioides difficile*.

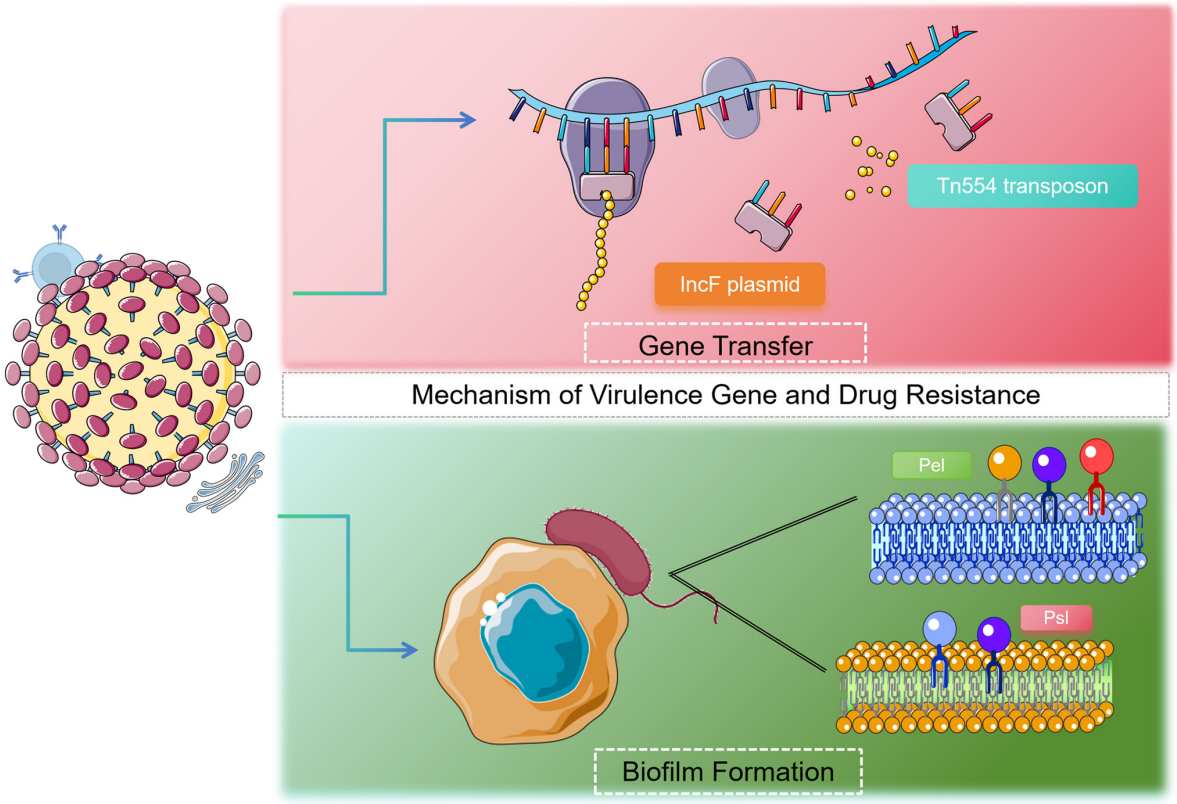


Figure 2. Mechanisms underlying the association between virulence genes and drug resistance. Tn554 (staphylococcal transposon 554), IncF (incompatibility group F plasmid), Pel (Pel polysaccharide synthesis locus), Psl (Pseudomonas surface polysaccharide locus). Tn554 and IncF act as mobile genetic elements that co-transfer virulence and antibiotic-resistance genes, accelerating spread. Pel and Psl encode exopolysaccharides needed for *Pseudomonas aeruginosa* biofilm formation; once the matrix is built, antibiotics penetrate poorly and resistance determinants are amplified within the protected community.

toxins and designed small-molecule inhibitors that mimic the toxin receptor-binding site (178). Preclinical studies in hamster models have shown that these inhibitors effectively blocked the binding of toxins to intestinal epithelial cells, and decreased the inflammatory response and disease mortality (72,178,179,181). Long-term use of the inhibitor did not induce the emergence of drug-resistant strains of *C. difficile*, which brings hope for clinical treatment (178).

In terms of adhesin targets, researchers have screened peptide drugs that interfere with the interactions between *S. pneumoniae* surface adhesins and host cell receptors (179,182,183). By competitively binding key adhesin sites, these peptides prevent bacterial colonization of the respiratory epithelium to prevent infection (179). Preclinical studies in mouse models of nasopharyngeal colonization have shown that treatment with peptides rapidly reduced S. pneumoniae load and airway inflammation without selecting for resistant mutants (Table VI) (179,182).

Optimization of combination therapy. Based on the association between virulence gene regulation and resistance mechanisms, therapeutic strategies targeting virulence pathways have emerged as promising alternatives to conventional antibiotic therapy (Fig. 2), which illustrates transposon and plasmid transfer linking virulence with resistance, *Pel* and *Psl* driven biofilm tolerance, and inhibitor based strategies targeting toxin or adhesin pathways to block infection without promoting resistance. For example, small-molecule inhibitors targeting bacterial toxins have demonstrated efficacy in preclinical models (72,178,183). The compound MI-888 disrupts the binding of *C. difficile TcdA/B* toxins to host receptors by mimicking the carbohydrate moiety of their target, markedly decreasing mortality in hamster models without inducing resistance mutations (178,184). Peptide Pep-19 blocks *S. pneumoniae* FnbA adhesin binding to host fibronectin, decreasing bacterial colonization when combined with amoxicillin in phase II trials (179,185). For biofilm-associated infection, alginate lyase enhances tobramycin penetration into *P. aeruginosa* biofilms, achieving a notable increase in bacterial clearance compared with monotherapy (72). These approaches leverage virulence-specific vulnerabilities to minimize collateral resistance induction, in contrast to traditional antibiotics, which target conserved bacterial machinery.

The combination of antibiotics and anti-virulence substances is common and effective for the treatment of infection. In the treatment of *H. pylori* infection, traditional single-antibiotic therapy faces a high drug resistance rate. Studies have found that a combination of amoxicillin and anti-virulence peptides, which inhibit the virulence of *H. pylori*, improves the therapeutic effect (183,186,187). Anti-virulence peptides enhance the penetration and bactericidal ability of amoxicillin to the *H. pylori* cell wall by interfering with the function of the bacterial cell membrane and inhibiting the secretion of virulence factors such as urease. Clinical studies have shown that combined treatment had a higher eradication rate, lower recurrence rate and

reduced drug-resistant strain isolation compared with monotherapy, which demonstrated the superiority of the combined treatment (183,188).

In addition, for biomembrane-associated infection, such as chronic pulmonary infection caused by *P. aeruginosa*, the combination of bioactive enzymes that can destroy the biofilm structure and sensitive antibiotics has synergistic potential (189). Biofilms, structured bacterial communities encased in a protective extracellular polymeric matrix, are resistant to antibiotics and immune clearance (190). Disruption of EPS components (such as alginate in *P. aeruginosa*) with enzymes (such as alginate lyase) enhances antibiotic penetration, offering a synergistic approach to treat chronic infection (191). Biological enzymes break down the EPS of biofilms and open penetration channels for antibiotics, exposing the bacteria to drug attack (192).

Experimental studies have shown that compared with single antibiotic treatment, combination therapy notably increases the bacterial clearance rate, improves lung inflammation indicators and delays the emergence of drug-resistant bacteria, providing novel ideas for the treatment of chronic refractory infection (72,193) (Table VI).

Clinical translation challenges

Off-target effects and resistance risks of anti-virulence drugs. Targeting host-pathogen interactions risks disrupting host physiology, as seen in therapies against SARS-CoV-2 and HIV-1. For example, ACE2 inhibitors interfere with the renin-angiotensin system, causing a rise in blood pressure in rhesus macaques (194). This dual role of ACE2 as a viral receptor and a regulator of blood pressure highlights the delicate balance between antiviral efficacy and host safety. To mitigate such off-target effects, researchers are developing lung-targeted nanocarriers to confine drug distribution to the infected tissue, thereby minimizing systemic exposure (170,194).

A similar challenge emerges in antiviral therapies that target variable viral proteins. The broadly neutralizing antibody VRC01 against the HIV-1 *env* protein selects for hypermutated variants with a high resistance frequency *in vitro*, driven by the high mutation rate in the V3 loop and glycan shield regions (44). This mirrors the challenge in bacterial anti-virulence strategies, where agents such as the antimicrobial peptide P113 face termination due to host toxicity. P113 disrupts *P. aeruginosa* biofilms by targeting polysaccharide synthesis locus (Psl) polysaccharides but induces hemolysis due to sequence similarity with human phosphatidylserine (169). Similarly, the *S. aureus hla* inhibitor RU-58 demonstrates a trade-off between efficacy and safety; while notably decreasing skin necrosis in mice, it binds host Kv1.3 channels, triggering arrhythmias at high doses (176,177).

These examples, spanning viruses, bacteria and hosts, reveal a common paradox in anti-virulence design: Targeting conserved virulence mechanisms often intersects with key host pathways, while targeting variable pathogen epitopes invites rapid resistance. Whether through nanocarrier-mediated spatial confinement for ACE2 inhibitors or multi-epitope vaccination for HIV-1, solutions must address both the species-specific vulnerabilities of pathogens and the evolutionary pressures they exert.

Standardization and clinical applicability of virulence gene detection. Accurate and rapid detection of virulence genes in clinical samples is key for precision medicine; however, there are hurdles in assay standardization and technical consistency (195). For example, detecting the *dosR* gene in *M. tuberculosis*, which governs the bacteria dormancy and reactivation within granuloma, requires reliable quantification in blood or sputum specimens (195). However, existing techniques, such as quantitative (q)PCR and nanopore sequencing, produce inconsistent outcomes. qPCR may miss *dosR* transcripts in hypoxic environments where bacterial metabolism slows, while nanopore sequencing, despite its sensitivity to low-abundance transcripts, exhibits variability in library preparation and base-calling accuracy (196). Similar issues affect the detection of other pathogen virulence genes. For example, identifying the *PVL* gene in *S. aureus* often yields discordant results between PCR and next-generation sequencing, with discrepancies reported in a notable proportion of clinical isolates due to primer mismatch, contamination during extraction and inconsistent positive-result thresholds (111). In antifungal diagnostics, detecting the *ssa1-ERG11* virulence-resistance module of *C. auris* via point-of-care tests risks false positives in a notable number of cases because of cross-reactivity with non-pathogenic species (195).

These challenges have direct implications for clinical decision making. In bloodstream infection caused by *K. pneumoniae*, delayed or inaccurate detection of the *rmpA* virulence gene can postpone combination therapy, potentially increasing the mortality risk (13,127). To overcome these obstacles, international initiatives aim to establish unified standards for virulence gene assays. The World Health Organization has promoted certified reference strains for *M. tuberculosis* and *S. aureus* to facilitate interlaboratory calibration (17,195). Researchers are also exploring multimodal approaches, combining qPCR for rapid screening with next-generation sequencing for confirmation, a strategy that notably decreases *P. aeruginosa* virulence gene detection errors (169,170). Additionally, AI-driven models show promise in reconciling platform-specific biases, such as correcting nanopore sequencing errors when analyzing hypervariable genes, including HIV-1 *env* (196,197). However, resource-limited regions face barriers due to a lack of standardized reagents and trained personnel (14,91). While emerging technologies, such as CRISPR-based specific high-sensitivity enzymatic reporter unlocking assays, offer potential for point-of-care applications, their widespread adoption requires development of globally consistent performance metrics (198,199). Bridging the gap between technological innovation and clinical utility requires interdisciplinary efforts.

Inadequate coverage of virulence gene polymorphisms in multivalent vaccines. The high variability of viral proteins, such as the *env* gene products of HIV-1, poses a challenge to targeted therapies and vaccine efficacy (42). For HIV-1, the broadly neutralizing antibody VRC01 targets the CD4-binding site of gp120; however, the virus rapidly evolves escape variants through mutations in the hypervariable V3 loop of the *env* gene, such as the addition of N160K glycosylation sites, which decreases antibody binding efficiency (41). *In vitro* studies have shown a high resistance frequency to VRC01, and a 2023 phase III trial revealed that the majority of patients developed

resistant strains following monotherapy, underscoring the need for multi-epitope strategies (45,200). This phenomenon is not exclusive to HIV-1 infection. Influenza vaccines designed to target the HA protein frequently fail to neutralize strains with antigenic drift, such as mutations in the HA1 domain of H3N2 viruses (46). Similarly, under immune pressure, the RBD of the SARS-CoV-2 S protein undergoes evolution, as evidenced by the o variant (19,129). This highlights the widespread challenges posed by the variability of viral epitopes.

The common thread linking these challenges is the ability of pathogens to exploit genetic and structural plasticity to evade immune recognition. For vaccines, this means designing multivalent formulations that cover hypervariable virulence epitopes, a task requiring breakthroughs in epitope prediction algorithms and delivery systems (201). Just as HIV-1 *env* variability necessitates multi-epitope antibody cocktails, influenza and SARS-CoV-2 vaccine development must confront HA and RBD mutational hotspots (202). Emerging technologies, such as AI-driven epitope mapping and self-assembling nanoparticle platforms, hold promise for identifying conserved regions (such as the HA stem in influenza or the RBD base in SARS-CoV-2) while accommodating variant-specific epitopes (42). These approaches mirror the those used for HIV-1: Single-target strategies are inherently vulnerable to viral evolution, whereas multi-pronged designs that anticipate variability, whether through computational prediction or dynamic antigen presentation, may provide durable protection against evolving pathogens (42).

Conservation limitations of fungal targets: C. albicans EFG1. The development of broad-spectrum antifungal therapies is hampered by the limited conservation of virulence targets across different fungal species. An example is the transcription factor EFG1 in *C. albicans*, which is a key regulator of hyphal development and virulence (16). The small-molecule inhibitor EFG1-IN-1 demonstrates notable efficacy in mouse models of systemic candidiasis, markedly decreasing mortality rates by blocking EFG1-dependent gene expression and inhibiting hyphal transition (16,55). However, to the best of our knowledge, this success has not been observed in fungal pathogens. Comparative genomics has revealed that the DNA-binding domain of EFG1 exhibits marked sequence divergence in species such as *A. fumigatus* and *Cryptococcus neoformans*. These structural disparities prevent EFG1-IN-1 binding to orthologous proteins in non-*Candida* fungi, rendering the inhibitor ineffective against invasive aspergillosis and cryptococcal meningitis (82,153). This lack of conservation is not exclusive to the EFG1 protein. Numerous fungal virulence factors, such as cell wall synthases and iron acquisition protein, exhibit species-specific structural characteristics that impede the development of pan-fungal drugs (82).

The consequences of such variability extend beyond individual compounds. Clinical trials of novel antifungals often fail when testing against diverse fungal pathogens, despite promising results in single-species models. For example, drugs targeting the β-1,3-glucan synthase complex, a key cell wall component, show efficacy against *Candida* but have limited activity against *Aspergillus* due to differences in enzyme subunit composition (82,203,204). To overcome these challenges, researchers are exploring two strategies: Identifying highly conserved domains within functionally key proteins, such as the catalytic core of fungal kinases, and designing inhibitors that tolerate minor sequence variations and developing combination therapies that target multiple species-specific virulence pathways (203,204). However, both approaches face challenges. The former requires extensive structural biology and computational modeling to predict binding modes across diverse homologs, whereas the latter requires understanding of pathogen-specific virulence mechanisms, making the development of broad-spectrum antifungal agents challenging (203,204).

5. Future prospects

Technological innovation. Integration of multi-omics data remains challenging. For example, determining the association between transcriptomic changes in *C. albicans* hyphae with proteomic dynamics during host invasion is complicated by temporal mismatches. Emerging spatial transcriptomics and single-cell CRISPR screens offer solutions but require standardized workflows to avoid data bias (72). The organic integration of genomics, transcriptomics, proteomics and metabolomics is used to analyze the molecular characteristics of pathogens during infection. For example, by simultaneously monitoring the transcriptional changes in virulence genes during bacterial infection, the expression regulation of corresponding virulence proteins and the fluctuations in host cell metabolites, a more refined and comprehensive pathogenic network can be constructed, and the mechanisms of virulence genes can be accurately revealed (205). The development of spatiotemporal omics technology may facilitate real-time tracking of the spatiotemporal dynamic distribution and gene expression changes of pathogens in infected tissue and organs. Combination of high-resolution imaging and single-cell sequencing technology may demonstrate how virulence genes are activated in specific cell subsets at specific time points in different infection stages of the virus in the host cell, providing understanding of the microscopic process of viral infection and facilitating the research and development of targeted therapeutic drugs to accurately locate key links (197).

Spatial transcriptomics and AI-driven modeling are accelerating the translation of virulence gene insights into clinical applications. In *C. albicans* keratitis, spatial omics-guided delivery of voriconazole-loaded nanogels to *Sap2*-expressing hyphae at infection foci improves drug accumulation compared with systemic administration (206,207). Meanwhile, AI models such as AlphaFold2 predict virulence protein-host receptor interactions, like the binding of *S. pneumoniae* capsular polysaccharide (Cps) to complement C3b (C3b), with atomic precision. This not only accelerates target identification but also informs the design of multivalent vaccines that cover the majority of clinical serotypes (208). Preclinical studies have shown that such vaccines induce notably higher opsonophagocytic antibody titers than current polysaccharide vaccines (209,210).

The integration of multi-omics approaches with single-cell resolution may facilitate the study of virulence genes. For example, single-cell transcriptomics enables the real-time tracking of virulence gene dynamics during

infection. A previous study applied this technology to *Salmonella*-infected macrophages, revealing subpopulations of bacteria with upregulated *SPI-2* (virulence island) genes under host ROS stress, which is associated with enhanced intracellular survival and systemic dissemination (29,211). Similarly, spatial transcriptomics is used to map virulence gene expression within infected tissue. In a murine model of *C. albicans* keratitis, spatial omics has identified hypha-specific *SAP* protease hotspots at the corneal invasion front, guiding targeted antifungal therapy (67). CRISPR-based functional screens offer precise tools to determine virulence-immune interactions. A pooled CRISPR interference screen in *P. aeruginosa* revealed luxR family transcriptional regulator lasR (a quorum-sensing regulator) as a key node balancing biofilm formation and immune evasion, with knockout strains showing notably reduced resistance to neutrophil killing (72). These approaches can prioritize therapeutic targets in pathogen-specific contexts.

Interdisciplinary cooperation. Research on pathogen virulence genes may highlight the importance of interdisciplinary cooperation and a collaborative innovation research model in medicine, biology, computer science and other disciplines. With clinical experience, medical experts accurately identify key problems in infectious diseases and guide basic research. Biological researchers have explored the functions and regulatory mechanisms of virulence genes to identify potential therapeutic targets. Computer scientists use advanced algorithms and big data processing capabilities to overcome the problems associated with the analysis of large amounts of biological data and build accurate predictive models. For example, in the development of novel antibacterial drugs, pharmaceutical chemists have designed targeted small-molecule inhibitors based on the virulence protein structure revealed by biological research and computer simulations of the drug-target combination process to optimize the molecular structure. Clinical trials are conducted to verify the efficacy and safety of these novel therapies. Through close collaboration among researchers, these trials help to accelerate the translation of scientific research findings from the laboratory to clinical application, thereby improving patient well-being and enhancing global health (79).

The complexity of infectious disease research necessitates integration of medicine, computational science and engineering. AI-driven platforms can predict the transmission risk of hypervirulent-resistant clones with high accuracy (2). In drug development, QM/MD co-simulations have optimized the design of broadly neutralizing antibodies targeting conformational changes in HIV-1 Env proteins, achieving an increase in neutralization potency against o subvariants (42). The convergence of synthetic biology and microfluidics has revolutionized pathogen-host interaction models. For example, a lung-immune organ-on-a-chip integrates human alveolar epithelium, endothelial cells and primary neutrophils to dynamically simulate *A. fumigatus* invasion and capture hyphal penetration of vascular barriers. This model has revealed that hypoxia upregulates *SidA* expression, leading to a marked increase in fungal load (69-71). Such platforms may replace animal testing and accelerate the preclinical evaluation of anti-virulence therapies.

Applications. Precision therapies based on virulence gene profiling have already impacted clinical decisions. In *ESBL*-producing *E. coli* infection, strains carrying the *fimH-blaCTX-M* plasmid are associated with high carbapenem resistance (5,7). Phage therapy using the engineered phage ΦECM1, which is armed with CRISPR-Cas9 to target plasmid-encoded virulence-resistance modules, has demonstrated higher bactericidal efficiency than meropenem in *ex vivo* human plasma models (5,212). For viral pathogens, the mRNA vaccine mRNA-1273.214 targets six hypervariable mutations in the SARS-CoV-2 RBD, inducing neutralizing antibodies against the majority of circulating variants in phase III trials (19,213). Similarly, a *C. albicans SAP* protease conjugate vaccine decreased the incidence of invasive candidiasis in immunocompromised patients during phase III testing (18). In precision therapy, the synergistic combination of engineered phages and antibiotics exhibits potential. By using CRISPR to silence the quorum-sensing regulator gene *lasR* in *P. aeruginosa*, engineered phages weaken biofilm barriers, markedly enhancing the bactericidal efficiency of meropenem in chronic lung infection, with safety validated in sputum samples from patients with cystic fibrosis (212). Epigenetic editing tools such as CRISPR-dCas9 offer novel strategies against fungal infection (55,72). Targeted suppression of *EFG1* in *C. albicans* blocks hyphal transition, increasing survival rates in murine systemic infection models without observable off-target toxicity (55,159).

Smart diagnostics and early warning systems are reshaping global infectious disease surveillance. Portable nanosensor arrays functionalized with gold nanoparticles simultaneously detect *Vibrio cholerae* cholera toxin A-B subunit (*ctxAB*) and New Delhi metallo-β-lactamase-1 (*blaNDM*) resistance genes, achieving high sensitivity in wastewater samples, thereby enabling early cholera outbreak alerts (198,199). Furthermore, a blockchain-powered global virulence gene database has successfully traced the cross-border transmission of *E. coli* clones carrying *fimH-blaCTX-M* plasmids across Asia and Africa, providing a data-driven foundation for regional antimicrobial resistance policies (10).

Vaccine development is overcoming traditional limitations using cutting-edge technology. A broad-spectrum mRNA vaccine for *SARS-CoV-2*, designed to cover most known variants by integrating S protein mutation hotspots from o BA.5 and XBB.1.5, has demonstrated a notable increase in neutralizing antibody titers compared with conventional vaccines in phase II trials (19,214). For fungal pathogens, a polysaccharide-protein conjugate vaccine targeting immunodominant epitopes of *C. albicans SAP* proteases activates T helper 17 cell responses, substantially reducing invasive candidiasis incidence in phase III trials, particularly in immunocompromised patients (18,215).

These advances mark a shift from reactive treatment to precision intervention, underscoring the key role of multidisciplinary convergence in addressing global health challenges. By integrating frontier technologies, clinical needs and public health data, future research may transform personalized therapy, outbreak prediction and vaccine coverage to alleviate the global burden of infectious disease.

6. Conclusion

Notable achievements have been made in research on pathogen virulence genes. From a basic theoretical level, the diversity, classification and complex mechanism of virulence genes of bacteria, viruses, fungi and other pathogens have been revealed, which provides an understanding of how pathogens use these to invade the host, evade immunity and cause diseases. These studies provide a foundation for developing precision medicine (216-218). Although there are challenges, including research gaps such as the molecular mechanisms of cross-kingdom virulence gene transfer, the spatiotemporal regulation of virulence genes in complex host microenvironments, the lack of standardization in virulence-based precision diagnostics, technical bottlenecks (such as multi-omics data integration) and ethical considerations, pathogen virulence gene research may be facilitated by advancements in frontier technologies (such as multi- and spatial-temporal omics) and interdisciplinary collaboration. Virulence gene research has transitioned from mechanistic identification to clinical implementation, yielding tangible applications in targeted anti-virulence therapy, precision diagnostics and next-generation vaccines. Compounds such as MI-888 and Pep-19 demonstrate the feasibility of inhibiting pathogen-host interactions without selecting for resistance, while blockchain-enabled global databases tracking *fimH-blaCTX-M* plasmids have identified cross-continental transmission hotspots to inform antibiotic stewardship policies (10). Multi-omics-driven vaccine designs, such as the SARS-CoV-2 pan-variant mRNA vaccine, address the challenges of antigenic diversity and set a precedent for adaptive immunization strategies. These advancements underscore the potential of this field to transform infectious disease management by offering data-driven solutions to decrease morbidity and mortality from drug-resistant pathogens and enhance global health. Development of multi-omics joint analysis, space-time omics and other frontier technology may facilitate interdisciplinary collaborative pathogen virulence gene research to resist pathogen invasion and maintain global health.

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Authors' contributions

YC performed the literature review and wrote the manuscript. XW, CX and JH designed the study. LZ, PQ, DZ and WC conceived the study. SZ revised the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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