Research Article

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SMP Otolaryngology and Rhinology

"Mucosal Blocking" Strategy for Prevention and Control of Infectious Diseases

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Publication Dates

Received date: May 30, 2025

Accepted date: June 02, 2025

Published date: June 06, 2025

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Citation

Wei Dong, Na-Na Peng, Yuan Yuan Zhu, Xiao Hong Xie, Lixin Wen et al. (2025) "Mucosal Blocking" Strategy for Prevention and Control of Infectious Diseases, SMP J Otolaryngol Rhinol, 2: 1-9

Abstract

This study presents an innovative mucosal blocking strategy for the prevention and control of infectious diseases. The proposed approach utilizes genetically engineered subunit proteins as molecular blockers to establish a robust mucosal defense system, effectively preventing pathogenic microorganisms from penetrating the mucosal barrier. The feasibility of this strategy was experimentally validated through a FimA-sefA protein assay, which successfully demonstrated the inhibition of Salmonella adhesion to IPEC-J2 cells. This is an innovative "mucosal blocking" strategy for infectious disease prevention and control. The approach employs genetically engineered subunit proteins as molecular blockers, demonstrating broad-spectrum efficacy against diverse serotypes, genotypes, and mutant strains. By preventing viral invasion of target cells and inhibiting viral penetration through mucosal barriers, this strategy establishes a novel frontline defense mechanism at the mucosal interface, representing a significant advancement in viral disease prevention and control methodologies.

Keywords: Biosafety; infectious disease prevention and control; mucosal blockade; self-purification livestock house

Introduction

Urgent Need for Strategic Upgrades and Improvements in the Existing Biosafety System for Infectious Disease Control

The recent emergence of major infectious diseases, particularly African swine fever and COVID-19, has created unprecedented challenges to global health security, exposing critical gaps in biosafety protocols for both human and animal health protection [1]. Throughout the past century, anthropogenic environmental degradation has significantly intensified human-wildlife interactions, while accelerated global mobility has dramatically enhanced the transnational transmission of pathogenic microorganisms. Compounding these factors, unprecedented demographic expansion and industrialized animal agriculture have created an increasingly vulnerable host population, significantly amplifying the potential for pathogen propagation and disease emergence [2]. The stagnation in preventive strategies and control measures has precipitated a global crisis in infectious disease management, particularly in the containment of emerging viral pathogens.

The current biosafety system for infectious disease prevention and control focuses on disrupting the infectious process by targeting one or more of the three essential elements: the source of infection, the route of transmission, and susceptible animals. This systematic approach, encompassing spatial segregation, comprehensive disinfection protocols, and pathogen detection mechanisms, is designed to interrupt the chain of infection and mitigate disease dissemination.

While the existing biosafety framework has played a crucial role in mitigating infectious diseases, it reveals significant strategic limitations that necessitate substantial enhancement. Firstly, once pathogenic microorganisms reach the skin or mucosal surfaces of susceptible animals, there are no defense mechanisms in place to prevent them from breaching the mucosal barrier and invading the animal body.

Secondly, the existing biosafety framework permits critical spatiotemporal windows that enable environmental pathogens to access susceptible hosts, thereby establishing potential transmission pathways. Current isolation measures confront substantial challenges, including the interception of arthropod vectors and wildlife harboring pathogens, as well as the implementation of comprehensive disinfection protocols for all incoming biological materials, personnel, vehicles, and equipment prior to facility entry.

The effectiveness of isolation is also affected by the isolation space environment, the length of isolation time, and the disposal measures. Similarly, the antimicrobial performance of conventional disinfectants is substantially impacted by application frequency, contact time with surfaces, and various environmental parameters, particularly the presence of organic matter, ammonium compounds, and pH fluctuations. Spatial isolation protocols implement stringent movement restrictions on biological materials, personnel, vehicles, and equipment to prevent pathogen introduction through infected sources or mechanical vectors. Furthermore, the reliability of diagnostic outcomes is significantly influenced by multiple factors, including sampling methodologies, testing frequency, reagent quality, instrumentation precision, and procedural execution during detection processes.

Mucosal barrier protection employs receptor-blocking strategies to inhibit viral penetration through epithelial surfaces, thereby reinforcing the primary immunological defense against viral infections. As the body's first line of defense, the integumentary system and mucosal membranes provide essential physical and immunological barriers [3], effectively preventing the colonization and invasion of numerous pathogenic microorganisms.

While the quantity of pathogenic microorganisms simultaneously reaching epithelial surfaces is generally limited, mucosal membranes constitute a crucial defensive interface in preventing microbial infections in host organisms. Notably, specific pathogens, particularly viruses, possess the capability to establish infection through molecular interactions between their surface antigens and cellular receptors on mucosal surfaces.

Through receptor-mediated endocytosis, these viral pathogens gain intracellular access for replication and subsequent dissemination into systemic circulation via both hematogenous and lymphatic routes, effectively compromising the mucosal barrier and establishing systemic infection, as illustrated in Figure 1. The mucosal blocking strategy utilizes specific molecular inhibitors that competitively bind to epithelial cell surface receptors, thereby preventing viral attachment and cellular entry, as demonstrated in Figure 2.

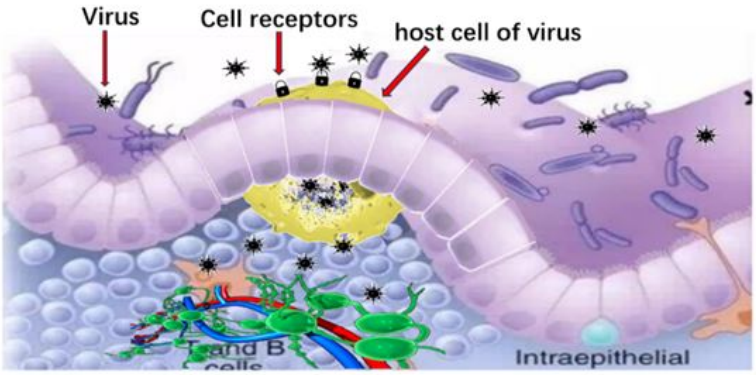

Figure 1: Mechanism of virus breakthrough of mucosal barrier

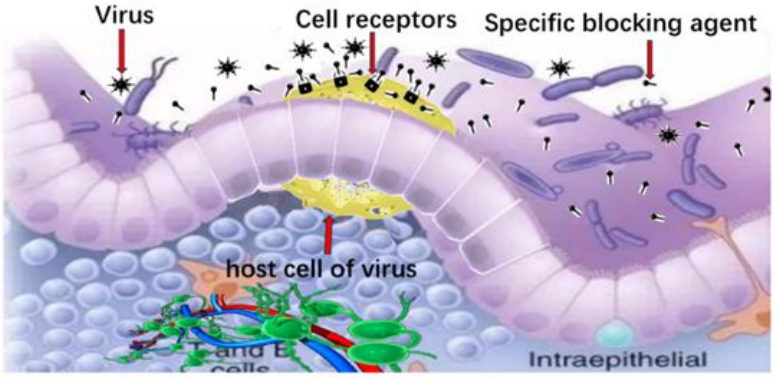

Figure 2: Mechanism of mucosal blocking

In summary, the mucosal blocking strategy and the use of subunit proteins expressed via DNA recombination technology represent powerful tools in modern vaccinology and infectious disease prevention. They offer a safe and effective means of inducing immunity and blocking pathogen entry at mucosal sites, with successful applications in vaccines for diseases like COVID-19, foot-and-mouth disease, rabies, and hepatitis B.

Second, the reactogenicity of subunit proteins is harnessed to develop diagnostic antigens [5] for detecting serum antibodies in animals. The scientific community widely regards the 21st century as the "century of genetic engineering," anticipating that it will propel significant advancements in the medical and biopharmaceutical industries. Despite the maturity of genetic engineering technologies today, their application has yet to reach its full potential. Obtaining the target gene sequences of known viruses has become relatively straightforward, as the cost of synthesizing these sequences is now quite low. Additionally, vector construction and transformation technologies are highly developed, and the methods for producing genetically engineered antigen proteins are advanced, with no significant capacity bottlenecks.

While genetic engineering subunit vaccines hold great promise, their practical applications often fail to fully address current demands. In contrast, emerging molecular biology diagnostics have seen broader adoption compared to serological diagnostics. The realization of a "century of genetic engineering" ultimately depends on its capacity to generate significant economic and social benefits.

To ensure effective blocking, the vaccine strain must closely match the circulating viral strain, as antibodies rely on this alignment to bind and block viral antigens. Given the extensive diversity of viral serotypes, genotypes, and mutant strains, this alignment is critical. The blocking agent is designed to target a specific receptor on host cells, which remains consistent across all strains of the same virus. As a result, the blocking agent can effectively prevent various serotypes, genotypes, and variant strains from infecting target cells. Moreover, compared to live attenuated vaccines, the

blocking agent demonstrates significant advantages ($p < 0.001$) in terms of safety, administration route, mechanism of action, action efficiency, targeting precision, resistance to viral variation, residue concerns, and prevention of vertical transmission, as detailed in Figure 3.

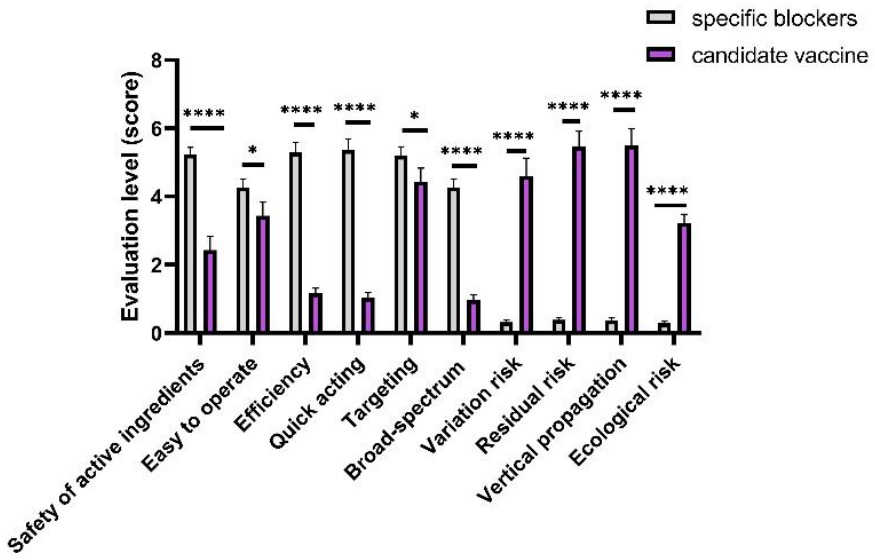

Figure 3: Comparison of blockers and vaccines (score on a five-point scale, with the highest score being 5 and the lowest score being 0)

Materials and Methods

Materials

Salmonella blockers are prepared in the laboratory.

2.2.1 Test of Blocking the Adhesion of *Salmonella* to Target Cells

Once IPEC-J2 cells reached approximately 90% confluence, FimA-sefA protein and recombinant bacteria were introduced and incubated at 37°C for 60 minutes. Subsequently, Salmonella bacteria were added at a multiplicity of infection (MOI) of 100 and incubated at 37°C for an additional 60 minutes. In the positive control group, only Salmonella bacteria were added. Following this, the cells were incubated overnight at 4°C with mouse anti-Salmonella LPS monoclonal antibody (diluted 1:50). After rinsing, the cells were incubated with FITC-conjugated sheep anti-mouse IgG (diluted 1:500) at 37°C for 60 minutes. Finally, the cells were stained with DAPI and visualized under a fluorescence microscope.

2.2.2 Animal Mucosal Blocking Test

Seventy-two specific pathogen-free (SPF) chicks were acquired and randomly allocated into six groups: an experimental group (receiving low, medium, and high doses of recombinant bacteria via oral administration), a negative control group (receiving NZ3900 via oral administration), a blank control group, and a positive control group. After three consecutive days of treatment, the chicks were challenged with Salmonella (1×10^9 CFU per chicken) administered orally. Clinical symptoms were monitored, and weight gain and mortality rates were recorded throughout the experiment.

Results

3.1 Cell Blocking Test Results

Immunofluorescence assay (IFA) confirmed that the FimA-sefA protein of Salmonella enteritidis and the recombinant bacteria expressing the FimA-sefA protein effectively blocked the adherence of Salmonella to IPEC-J2 cells, as illustrated in Figure 4.

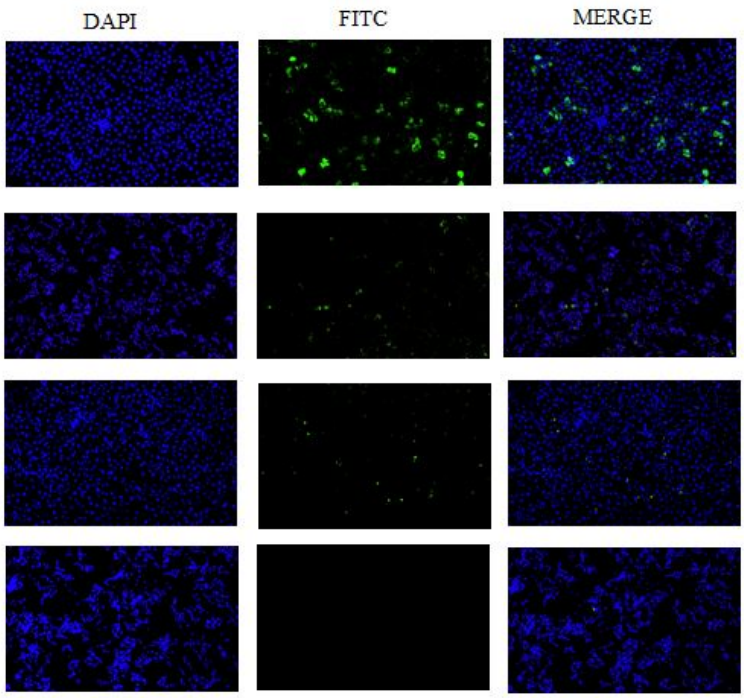

Figure 4: FimA-sefA protein and recombinant bacteria block the adhesion of Salmonella to IPEC-J2 cells

A: Positive control; B: FimA-sefA protein; C: recombinant bacteria; and D: negative control group

3.2 Results of animal challenge protection test

The positive control group exhibited the lowest weight gain, with a mortality rate of 16.6%. In contrast, the low-, medium-, and high-dose groups demonstrated a dose-dependent increase in body weight ($p < 0.001$), and no mortality was observed, as depicted in Figure 5.

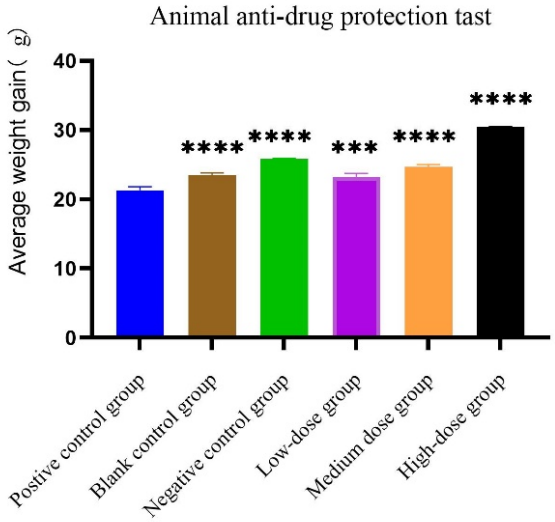

Figure 5: Blocking effect of recombinant *Lactococcus lactis* (**** $p < 0.0001$ and *** $p < 0.001$)

Discussion

This study introduces and substantiates the feasibility of mucosal blocking as a novel approach. We hypothesize that the blocking efficacy of recombinant bacteria is attributed to the specific interaction between the ligand-protein present on the bacterial surface and the corresponding receptor on target cells. This interaction facilitates the adherence of recombi-

nant bacteria to the cell surface, thereby creating a steric hindrance that effectively impedes the adhesion of Salmonella enteritidis to the target cells.

Mucosal blocking emerges as a promising strategy for combating African swine fever (ASF), a highly contagious disease that has caused widespread outbreaks across multiple countries. In the absence of an effective vaccine [6], current prevention and control efforts rely exclusively on biosecurity measures, which impose substantial financial and technological demands on the pig industry. This heavy burden has led to the decline of small and medium-sized breeding operations and family farms, fundamentally reshaping the structure of the pig industry. Despite rigorous implementation of existing biosecurity protocols, recurrent ASF outbreaks continue to inflict significant economic losses on certain pig farming enterprises [7]. The restricted cell tropism of African swine fever virus (ASFV) indicates that its infectivity is dependent on specific cellular receptors [8-10]. Current research has demonstrated that ASFV employs dual entry mechanisms to infect macrophages: macropinocytosis and clathrin-mediated endocytosis [11]. Notably, both pathways ultimately converge into the endocytic pathway, which requires precise spatial and temporal coordination of protein and lipid components on the endosomal membrane. This membrane reorganization process is fundamentally dependent on specific molecular interactions between viral antigens and endocytosis-associated receptors [12-15].

The novel coronavirus initiates infection by recognizing and binding to the ACE2 receptor on target cells within the human respiratory tract mucosa [16,17]. The proposed mucosal blockade strategy functions through competitive binding to ACE2 receptors, thereby preventing viral attachment. This innovative approach to COVID-19 prevention offers several distinct advantages: (1) It creates a biological "mask" at the mucosal surface by competitively inhibiting viral-receptor interactions, effectively preventing viral penetration through the mucosal barrier. (2) The strategy maintains its efficacy against emerging viral variants [18-22], as it targets the conserved host receptor rather than the mutable viral binding domain. (3) The use of small molecular protein blockers significantly reduces the potential toxicity associated with the viral S protein. (4) This approach may contribute to slowing viral evolution by reducing the selective pressure for receptor-binding domain mutations and minimizing opportunities for viral recombination.

In the era of globalization, the frequency of "black swan" events, particularly major emerging infectious disease outbreaks, has significantly increased, posing substantial threats to both human and animal health. Compounding this challenge is the accelerated mutation rate of pathogenic microorganisms, which renders vaccine development increasingly complex and time-intensive. Current biosecurity systems for infectious disease prevention and control exhibit critical limitations: they fail to establish defensive measures at the crucial entry points where pathogenic microorganisms (especially viruses) invade host organisms, instead relying on incremental improvements to existing tactical approaches. This conventional system not only incurs higher costs but also frequently results in unconventional and often impractical operational measures [23], while still falling short of achieving desired prevention and control outcomes. Consequently, the management of infectious diseases, particularly viral infections, has emerged as a pressing public health priority. In this context, the "mucosal blocking" strategy offers novel theoretical frameworks and innovative approaches to address these critical challenges.

Acknowledgments

We thank LetPub (www.letpub.com.cn) for its linguistic assistance during the preparation of this manuscript.

Author Contributions

Authors Wei Dong and Nana Peng have contributed equally to this work.

Competing Interests

Authors declare that they have no competing interests.

Funding

Development of Preventive Formulations for Blocking ASFV Virus Infection (2022xczx-416); Development of Blockers for Five Major Animal Diseases (Swine Pneumonia, Porcine Reproductive and Respiratory Syndrome, Avian Salmonellosis, Orf disease, Brucellosis) (2021kjc-js178).

Data and Materials Availability

All data are available in the main text or the supplementary

materials.

References

1. Luo YW (2020) The concept and thinking of biosecurity from the perspective of overall national security J.. Chongqing Social Sciences, 2020: 10.

2. Carlson CJ, Albery GF, Merow C, Trisos CH, Zipfel CM et al. (2022) Climate change increases cross-species viral transmission risk, J Nature, 607: 555-62.

3. Wang Tao, Tian Xinlei, Zhang Di, Li Na, Qian Aidong (2022) Research progress in probiotic bacteria modulating intestinal mucosal immunity, J Chinese Journal of Veterinary Science, 42: 2578-84.

4. Callaway E (2020) The race for coronavirus vaccines: a graphical guideJ.. Nature, 580: 576-7.

5. Wang Bao-kun,Ran Xiang-yang, Zhang Lin, Feng Zitan, Chen Xing'an et al. (2005) Diagnosis of Helicobacter pylori infection using mixed purified recombinant subunit antigens J Laboratory Medicine, 2: 136-9.

6. Wu K, Liu J, Wang L, Fan S, Li Z et al. (2020) Current State of Global African Swine Fever Vaccine Development under the Prevalence and Transmission of ASF in China. Vaccines (Basel), 8: 531.

7. Gaudreault NN, Madden DW, Wilson WC, Trujillo JD, Richt JA (2020) African Swine Fever Virus: An Emerging DNA Arbovirus. Front Vet Sci, 7:215.

8. Chen X, Zheng J, Liu C, Li T, Wang X et al. (2023) CD1d facilitates African swine fever virus entry into the host cells via clathrin-mediated endocytosis. Emerg Microbes Infect, 12: 2220575.

9. Netherton CL, Shimmon GL, Hui JYK, Connell S, Reis AL (2023) African Swine Fever Virus Host-Pathogen Interactions. Subcell Biochem, 106: 283-331.

10. Sánchez EG, Pérez-Núñez D, Revilla Y (2017) Mechanisms of Entry and Endosomal Pathway of African Swine Fever Virus. Vaccines (Basel), 5: 42.

11. Cuesta-Geijo MÁ, García-Dorival I, Del Puerto A, Urquiza J, Galindo I et al. (2022) New insights into the role of endosomal proteins for African swine fever virus infection. PLoS Pathog, 18: e1009784.

12. Wang G, Xie M, Wu W, Chen Z (2021) Structures and Functional Diversities of ASFV Proteins. Viruses, 13: 2124.

13. Gao Q, Yang Y, Luo Y, Chen X, Gong T et al. (2023) African Swine Fever Virus Envelope Glycoprotein CD2v Interacts with Host CSF2RA to Regulate the JAK2-STAT3 Pathway and Inhibit Apoptosis to Facilitate Virus Replication. J Virol, 97: e0188922.

14. Yang S, Miao C, Liu W, Zhang G, Shao J et al. (2023) Structure and function of African swine fever virus proteins: Current understanding. Front Microbiol, 14: 1043129.

15. Li Z, Chen W, Qiu Z, Li Y, Fan J et al. (2024) African Swine Fever Virus: A Review. Life (Basel), 12: 1255.

16. Li J, Lai S, Gao GF, Shi W (2020) The emergence, genomic diversity and global spread of SARS-CoV-2 Nature, 600: 408-18.

17. Liu K, Tan S, Niu S, Wang J, Wu L et al. (2021) Cross-species recognition of SARS-CoV-2 to bat ACE2J.. Proc Natl Acad Sci U S A, 118: e2020216118.

18. Khan A, Zia T, Suleman M, Khan T, Ali SS et al. (2021) Higher infectivity of the SARS-CoV-2 new variants is associated with K417N/T, E484K, and N501Y mutants: An insight from structural data. J Cell Physiol, 236: 7045-57.

19. Kim YJ, Jang US, Soh SM, Lee JY, Lee HR (2022) The Impact on Infectivity and Neutralization Efficiency of SARS--CoV-2 Lineage B.1.351 Pseudovirus. Viruses, 13: 633.

20. Gupta D, Sharma P, Singh M, Kumar M, Ethayathulla AS, Kaur P (2021) Structural and functional insights into the spike protein mutations of emerging SARS-CoV-2 variants. Cell Mol Life Sci, 78: 7967-89.

21. Starr TN, Greaney AJ, Addetia A, Hannon WW, Choudhary MC et al. (2021) Prospective mapping of viral mutations that escape antibodies used to treat COVID-19. Science, 371: 850-4.

22. Zhang L, Li Q, Liang Z, Li T, Liu S et al. (2022) The significant immune escape of pseudotyped SARS-CoV-2 variant OmicronJ.. Emerg Microbes Infect, 11: 1-5.

23. Huang Wenhua, Chen Fangzhou, Fan Cuihua, Yang Huawei, Zhao Zukai (2023) Implementation of Biosafety System in Pig Farms Against the Background of African Swine FeverJ.. Swine Industry Science | Swine Ind Sci, 40: 30-4.