Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Host development shapes resistance, mobility, and virulence networks in the gut microbiome of wild Erinaceus amurensis

Xiuqi Jia
Northeast Forestry University
Xiang Li
Northeast Forestry University
Xinru Lv
Northeast Forestry University
Yi Li
Northeast Forestry University
Zhensheng Liu
Northeast Forestry University
Hongliang Chai
Hongliang_Chai@hotmail.com
Northeast Forestry University

Research Article

Keywords: Erinaceus amurensis, ARGs, MRGs, BRGs, VFs, metagenomics

Posted Date: March 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8897134/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Antimicrobial resistance (AMR) is increasingly recognized as an ecological problem extending beyond clinical and agricultural systems. Wildlife inhabiting human-influenced environments may integrate resistance signals from multiple environmental sources, yet how resistance-related functions are structured across host development remains poorly understood. Here, we used deep metagenomic sequencing to characterize antibiotic resistance genes (ARGs), metal and fungicide resistance genes, mobile genetic elements (MGEs), and virulence factors in the gut microbiota of wild *Erinaceus amurensis* collected from Northeast China, including male, female, and fetal individuals. We found pronounced developmental stratification of resistance-associated functions. Adult hedgehogs harbored expanded and interconnected networks of ARGs and MGEs, whereas fetal samples exhibited a markedly simplified resistance repertoire and distinct virulence-associated profiles. ARG enrichment in adults was dominated by multidrug, MLS, and β-lactam resistance and strongly co-occurred with transposases, integrases, and insertion sequences, indicating a central role for horizontal gene transfer. In parallel, metal and fungicide resistance genes were enriched in adults, supporting co-selection under long-term environmental chemical exposure. In contrast, fetal microbiomes showed limited resistance diversity but relative enrichment of secretion and adhesion-related virulence factors. Together, these results indicate that resistance, mobility, and virulence functions in wildlife gut microbiomes are not vertically fixed but are progressively shaped by host development and environmental exposure. Our findings highlight wild mammals as ecological integrators of environmental resistomes and underscore the importance of incorporating wildlife into antimicrobial resistance surveillance within a One Health framework.

Introduction

Antimicrobial resistance (AMR) has become a major threat to global public health and ecosystem stability and is now recognized by the World Health Organization as a “slow pandemic.” Beyond clinical and agricultural settings, sustained human activities have released antibiotics, heavy metals, and biocides into natural environments over long time scales. These pressures have reshaped microbial communities and driven the persistence and spread of resistance genes (RGs), leading to the formation of a large and heterogeneous environmental resistome. Growing evidence shows that resistance genes originating from environmental reservoirs can enter clinical pathogens through multiple ecological routes, contributing to the emergence of hospital-acquired resistance. Despite this risk, resistance dynamics in natural ecosystems, especially those involving wildlife, remain poorly characterized.

Within the One Health framework, wildlife is increasingly viewed as a biological indicator of environmental transmission of resistance determinants[1]. A wide range of wild animals, including birds and small mammals, have been reported to carry diverse antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), suggesting their potential role in mediating gene exchange across environmental, animal, and human compartments[2, 3]. Notably, methicillin-resistant Staphylococcus aureus and other multidrug-resistant bacteria have been detected in European hedgehogs, with evolutionary origins that may predate the clinical use of antibiotics[4]. These findings point to a long-standing association between wildlife hosts, microbial communities, and resistance traits.

The Amur hedgehog (*Erinaceus amurensis*) is widely distributed across Northeast Asia and occupies habitats that range from forests to agricultural and peri-urban landscapes. These habitats overlap extensively with areas of human activity. As an omnivorous and nocturnal species, *E. amurensis* frequently contacts soil, surface water, and diverse food sources, all of which are key reservoirs of environmental microbes. This ecological niche places the species at the interface between natural ecosystems and anthropogenic pressures, making its intestinal microbiome a suitable system for assessing environmental exposure to antibiotics, heavy metals, and associated resistance factors.

Horizontal gene transfer plays a central role in the dissemination of resistance genes, with mobile genetic elements acting as key mediators. The co-occurrence of plasmids, integrons, and transposons is often associated with increased transfer potential of ARGs and their movement from environmental reservoirs into pathogenic bacteria[5]. In parallel, long-term contamination by heavy metals and biocides can impose co-selection pressures that favor the joint enrichment of antibiotic resistance genes and metal or biocide resistance genes. These processes may increase the stability and persistence of resistance networks in natural microbial communities[6, 7]. However, in wild mammals, the joint distribution of resistance genes, virulence factors, and mobile genetic elements has rarely been examined at the metagenomic level.

Host-related factors further complicate these dynamics. Habitat alteration, Spatial and temporal axes, and physiological condition are known to influence gut microbiome structure and function[8–10], yet their combined effects on resistance and virulence gene networks in wildlife remain unclear. In particular, fetal and juvenile stages may exhibit distinct patterns due to immature immune systems and limited environmental exposure, but systematic evidence from natural populations is lacking.

In this study, we analyzed wild *E. amurensis* individuals from Heilongjiang Province in Northeast China, including females, males, and fetal samples. Using high-depth metagenomic sequencing, we integrated annotations from CARD, BacMet, VFDB, and multiple mobile genetic element databases to characterize antibiotic resistance genes, metal and fungicide resistance genes, virulence factors, and mobile genetic elements in the hedgehog gut microbiome. We compared their composition, abundance, and association patterns across sex and developmental stages. By doing so, we aimed to clarify how host traits shape resistance and virulence networks in a wild mammal and to provide baseline data for environmental antimicrobial resistance surveillance and ecological risk assessment.

Results

Developmental stage shapes gut antimicrobial resistance functions

Metagenomic analysis revealed widespread antimicrobial resistance–related functions in the gut microbiota of *Erinaceus amurensis*. A total of 2,703,808 reads were annotated to the KEGG pathway "Drug resistance: antimicrobial." Read abundance followed a clear developmental pattern, with the highest counts observed in adult males (1,537,592 reads), followed by adult females (1,157,720 reads), while fetal samples showed markedly lower levels (8,496 reads) (Fig. 1a–d). This pattern indicates that antimicrobial resistance–associated functional potential increases with host development and remains limited in fetal gut communities.

Annotation against the CARD database identified 624 antibiotic resistance genes (ARGs) spanning 21 antibiotic classes, including aminoglycosides, β-lactams, macrolide–lincosamide–streptogramin (MLS), multidrug resistance, glycopeptides, and tetracyclines (Fig. 1e; Table S1). Among these, 318 ARGs were shared across all groups, while adult males, adult females, and fetuses harbored 85, 10, and 1 unique ARGs, respectively (Fig. 1l–o). Resistance mechanisms were dominated by antibiotic efflux, followed by target alteration and antibiotic inactivation, with efflux genes prevailing across all developmental stages (Fig. 1p).

At the class level, multidrug, MLS, glycopeptide, tetracycline, and peptide resistance genes showed the highest relative abundance, with a consistent gradient of M > FM > FE (Fig. 1f–h; Table S2). Nucleoside resistance genes were detected exclusively in adult individuals, suggesting an expansion of resistance breadth during host maturation. Adult males showed enrichment of several dominant ARGs, including *ermF*, *ccrA*, *adeK*, *vanXO*, *oprN*, *tet(Z)*, and *blaZ*, reflecting broad-spectrum resistance potential (Fig. 1m).

Non-metric multidimensional scaling (NMDS) and ANOSIM analyses demonstrated clear separation of fetal samples from adult groups ($R = 0.608$, $P = 0.001$; stress < 0.05), while male and female adults clustered closely (Fig. 1i–j). Consistent with this pattern, Kruskal–Wallis tests identified significant enrichment of *evgS* (multidrug class) and *lmrD* (MLS class) in adults, whereas *oleC* (MLS class) was reduced in fetuses (Fig. 1k), indicating enhanced efflux and regulatory resistance mechanisms with development.

Mobile genetic elements co-enrich with resistance genes in adult guts

A total of 298 mobile genetic element (MGE) subtypes belonging to six major classes were detected across all samples (Table S3). Transposases (176 subtypes) and recombinases (70 subtypes) were the dominant components. Adult males harbored the highest total number of MGEs (279) and unique subtypes (49), compared with adult females (244 total, 16 unique) and fetuses (134 total, 1 unique) ($P < 0.01$). Ninety-nine MGEs were shared among all groups (Fig. 2b).

The most abundant MGEs included transposases, recombinases, integrases, IS3, IS1380, and Tn3 elements, all of which are known mediators of gene rearrangement and horizontal transfer (Fig. 2a). NMDS and ANOSIM analyses revealed significant developmental-stage–dependent differences in MGE composition ($R = 0.595$, $P = 0.001$; stress = 0.089), with adult samples clustering together and fetal samples forming a distinct group (Fig. 2d–e). Abundances of transposases, recombinases, and integrases differed significantly across groups ($P < 0.001$) (Fig. 2f).

Co-occurrence network analysis showed strong positive correlations between multidrug resistance genes and integrases as well as IS3 elements ($r > 0.6$) (Fig. 2c). This pattern suggests that the enrichment of MGEs in adult gut microbiota may facilitate the horizontal transfer and persistence of resistance genes.

Metal and fungicide resistance genes reflect cumulative environmental exposure

BacMet annotation identified 493 resistance-related genes, including 213 metal resistance genes (MRGs, 45.4%), 204 fungicide resistance genes (BRGs, 43.4%), and 52 genes conferring dual resistance $\mathrm{MRGs/BRGs}, 11\%$. Overall abundance followed a consistent gradient of M > FM > FE (Fig. 3a–c; Table S4).

Adult males harbored the highest diversity of MRGs (276 types, 16 unique), followed by adult females (262 types, 3 unique) and fetuses (197 types). Dominant MRGs in males included *ffurA*, *pbrR*, *golT*, *mdrL/yfmO*, *corS*, *chrI*, *merB*, *cueA*, *hupE*, *cutO*, and *ycnJ*, conferring resistance to multiple heavy metals such as Fe, Pb, Cu, Zn, Cr, Hg, Ag, and Ni (Table S5). Female samples were characterized by enrichment of *aioS*/*aoxS*, *ncrC*, and *ziaR*, primarily associated with Zn and Ni resistance pathways (Fig. 3j–l, 3p). NMDS and ANOSIM analyses confirmed significant differentiation in MRG composition among developmental stages ($R = 0.569$, $P = 0.001$; stress < 0.05) (Fig. 3d–e).

A total of 263, 244, and 192 BRGs were detected in adult males, adult females, and fetuses, respectively. Adult males uniquely harbored 19 BRGs, including *vmeA*, *vmeD*, *adeA*, *farB*, *mdrL/yfmO*, and *ttgT*, which are involved in resistance to surfactants and organic fungicides. Female samples were dominated by *vmeP* and *qacG*, while fetal samples showed limited diversity (Fig. 3m–o, 3q; Table S6). Multidimensional scaling analysis revealed significant group-level separation ($R = 0.586$, $P = 0.001$; stress = 0.094) (Fig. 3g–h). Several genes associated with chemical stress response and detoxification, including *merA*, *evgS*, *galE*, *oxyR-kp*, and *mdeA*, showed significant intergroup differences, while *fabL*/*ygaA* and *bcr* were highly enriched in adults ($P < 0.001$) (Fig. 3i).

Virulence factors and pathogen-associated profiles vary with development

VFDB annotation identified four major categories of virulence factors: defensive, nonspecific, offensive, and regulatory. The most prevalent functional types included antiphagocytosis, serum resistance, secretion systems, adherence, invasion, and toxins (Fig. 4c). In total, 506 virulence factors were detected, with adult males, adult females, and fetuses harboring 124, 14, and 3 unique factors, respectively (Fig. 4a; Table S7).

Functional composition differed across stages. Fetal samples showed relative enrichment of secretion systems (42%) and adhesion-related factors (35%), whereas adult samples were dominated by defensive functions such as antiphagocytosis and serum resistance (Fig. 4i). PHI-base annotation further

identified 4,547 genes linked to 187 pathogens and 216 host species (Table S8). Predominant pathogens included *Salmonella enterica*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Fig. 4d), with hosts primarily mammals and poultry (Fig. 4e).

Most pathogen-associated profiles were shared across groups, although unique taxa were detected only in adult males (Fig. 4b). NMDS and ANOSIM analyses confirmed significant compositional differences among developmental stages (R = 0.621, P = 0.001; stress < 0.05) (Fig. 4f–g). Several virulence-associated systems, including LOS (*Haemophilus*), T6SS (*Shigella sonnei*), polar flagella (*Aeromonas hydrophila*), capsule (*Klebsiella pneumoniae subsp.*), and T3SS (Yersinia), showed significant stage-specific differences (Fig. 4h), indicating functional divergence in pathogen interaction potential during host development.

Discussion

This study provides a metagenomic view of how antimicrobial resistance, genetic mobility, and virulence-related functions are organized across developmental stages in the gut microbiota of wild *Erinaceus amurensis*. Rather than forming a static functional background, these traits display clear developmental stratification, with adult individuals harboring expanded resistance and mobility networks, while fetal samples retain a simplified resistance repertoire and distinct virulence-associated features. This pattern suggests that resistance-related functions in wildlife gut microbiomes are largely shaped after birth and progressively reorganized through environmental exposure, rather than being stably established through vertical transmission.

The resistance gene profiles observed in adult hedgehogs were dominated by multidrug, MLS, and β-lactam resistance categories, which are commonly associated with environments influenced by human activity. The sampling area represents an agroforestry transition zone where agricultural inputs, livestock production, and domestic wastewater converge. Such settings provide continuous, low-level exposure to antibiotics and heavy metals, creating a persistent environmental resistance background. Under these conditions, wildlife gut microbiota are likely subjected to long-term selective pressure that favors the accumulation and maintenance of resistance genes[11, 12]. The absence of nucleoside resistance genes in fetal samples further supports the idea that resistance diversification reflects exposure history rather than maternal inheritance, consistent with observations in other wild vertebrates where resistance traits are predominantly acquired from the environment[13–15].

Mobile genetic elements appear to play a central role in stabilizing these resistance patterns. The strong concordance between ARG and MGE abundance across developmental stages suggests that horizontal gene transfer contributes to the persistence and spread of resistance genes within the gut ecosystem. Enrichment of transposases, integrases, and insertion sequences in adult individuals provides a molecular framework that may facilitate gene rearrangement and exchange across bacterial taxa. Similar coupling between resistance and mobility has been reported in human wastewater, livestock systems, and polluted aquatic environments[16–18], indicating that this mechanism is not host-specific but reflects a broadly conserved evolutionary strategy. While our analyses are based on short-read assemblies, future long-read sequencing will be required to resolve the physical linkage between ARGs and MGEs and to confirm their co-localization on transferable genetic elements.

In parallel, the enrichment of metal and fungicide resistance genes in adult individuals points to the influence of long-term chemical exposure on gut microbial function. Heavy metals such as Hg, Pb, Cu, and Zn, together with biocides and fungicides, can enter wildlife through water, soil, and diet, imposing selective pressures that extend beyond antibiotic use. The co-occurrence of metal and fungicide resistance genes with ARGs supports the role of co-selection in maintaining resistance traits under multi-stressor conditions. Differences observed between adult and fetal groups suggest that chemical stress responses are developmentally structured and may interact with host physiology, although the specific contribution of reproductive state or early-life stress cannot be fully resolved in this study.

Virulence-associated functions also exhibited clear stage-specific patterns. Adult gut microbiota were enriched in defensive traits such as antiphagocytosis and serum resistance, which are commonly associated with long-term host–microbe coexistence. In contrast, fetal samples showed relatively higher representation of secretion systems and adhesion-related factors. This contrast may reflect differences in immune maturity, niche availability, and early colonization dynamics rather than increased pathogenic risk per se. Together, these findings suggest that resistance and virulence do not increase in parallel during development but are shaped by different ecological and host-mediated constraints, resulting in a dynamic balance within the gut microbiome.

Taken together, our results indicate that wild mammals inhabiting human-influenced landscapes can act as integrators of environmental resistance signals rather than passive reservoirs. The developmental structuring of resistance, mobility, and virulence functions highlights the importance of host life stage in shaping microbial functional potential. Within a One Health context, these findings support the inclusion of wildlife species from human–environment interfaces in antimicrobial resistance surveillance. At the same time, they underscore the need for integrative approaches that combine genomic resolution, ecological context, and host biology to better understand resistance evolution in natural systems.

Methods

Study area and sample collection

Wild Erinaceus amurensis individuals were collected from Sishan Forest Farm, Jidong County, Heilongjiang Province, China (131°54'47"E–131°08'18"E, 44°58'53"N–44°51'12"N), a region characterized by a forest–farmland mosaic landscape. Sampling was conducted between April and July 2023 using live-capture cages. A total of 13 individuals were obtained, including five males, five females, and three fetuses. Samples were grouped according to sex and developmental stage as male (M), female (FM), and fetal (FE) groups.

Fresh fecal material was collected from the mid-intestinal section of each individual using non-invasive procedures[19]. Samples were immediately placed on dry ice, transported to the laboratory, and stored at − 80°C until processing. All sampling procedures were approved by the Heilongjiang Provincial Forestry

Authority and complied with relevant wildlife protection regulations and ethical guidelines.

DNA extraction and library construction

Total genomic DNA was extracted using the Mag-Bind® Soil DNA Kit (Omega Bio-tek, USA) following the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and integrity was verified by agarose gel electrophoresis. DNA was fragmented to an average size of approximately 350 bp using a Covaris M220 ultrasonicator. Paired-end sequencing libraries were prepared using the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, USA), followed by adapter ligation, PCR amplification, and magnetic bead purification.

Metagenomic sequencing and data processing

Libraries were sequenced on an Illumina NovaSeq 6000 platform (Illumina, USA) in paired-end mode (PE150). Raw reads were quality-filtered using fastp (v0.20.0), removing low-quality reads ($Q < 20$), reads containing ambiguous bases, and adapter contamination. Reads shorter than 50 bp after trimming were discarded. Host-derived reads were removed by alignment to the host genome using BWA (v0.7.9a).

High-quality reads were assembled de novo using MEGAHIT (v1.1.2), retaining contigs ≥ 300 bp. Gene prediction was performed using Prodigal and MetaGene, and open reading frames ≥ 100 bp were translated into amino acid sequences. All predicted genes were clustered using CD-HIT (v4.6.1) at 90% sequence identity and 90% coverage to generate a non-redundant gene catalog. The longest sequence in each cluster was retained as the representative gene. Clean reads were mapped back to the non-redundant gene set using SOAPaligner (v2.21) with a minimum identity of 95%, and gene abundance was calculated and normalized as previously described[20–26].

Functional and resistance gene annotation

Non-redundant protein sequences were aligned against multiple databases using DIAMOND (v0.8.35; E-value ≤ 1×10^{-5})[27]. Functional annotation was performed against KEGG, while antibiotic resistance genes were identified using CARD and ARDB. Metal and fungicide resistance genes were annotated using BacMet. Mobile genetic elements, including insertion sequences, transposons, integrons, plasmids, and phage-associated elements, were identified using a curated MGE database. Virulence factors were annotated using VFDB, and pathogen–host interaction genes were identified using PHI-base. For each gene, the best alignment hit was retained as the final annotation. Functional category abundances were calculated by summing the abundances of annotated genes.

Statistical analysis

Differences among groups were assessed using one-way ANOVA or Kruskal–Wallis tests, depending on data distribution, with significance defined as $P < 0.05$. Community-level differences in ARGs, metal and fungicide resistance genes, mobile genetic elements, virulence factors, and pathogen-associated profiles were evaluated using non-metric multidimensional scaling (NMDS) and analysis of similarities (ANOSIM). NMDS ordinations were considered reliable when stress values were < 0.2, and group separation was considered significant when ANOSIM $R > 0$ and $P < 0.05$[28, 29]. All analyses and visualizations were performed in R (v4.2.2).

Declarations

Ethics and biosafety statement

Ethics Statement: Sample collection for this study was approved by the Experimental Animal Ethics Committee of Mudanjiang Normal University (Approval No.: 2026-011), and all participants signed informed consent forms. Animal experiments were approved by the Experimental Animal Ethics Committee of Mudanjiang Normal University (Approval No.: 2026-011) and conducted in accordance with NIH guidelines for laboratory animals.

Biosafety Statement: Experimental procedures were conducted in an Animal Biosafety Level 2 (ABSL2) laboratory in compliance with the Regulations on Biosafety Management of Pathogenic Microorganism Laboratories in China.

Research funding

This study is supported by Young Academic Backbone Project of Mudanjiang Normal University (MNUQN202305),Prevention and Control of Emerging and Major Infectious Diseases-National Science and Technology Major Project (2025ZD01900100), National Natural Science Foundation of China (32500447), Major Project of Guangzhou National Laboratory (GZNL2023A01001),Heilongjiang Provincial Natural Science Foundation Joint Fund Key Project: Research into AI and eDNA-based High-Precision Monitoring and Intelligent Prediction Technologies for the Aquatic Ecosystem of Mirror Lake (ZL2025C004),Sub-project under the National Key Research and Development Programme: Establishment of a Common Black-striped Field Mouse Population (2023YFF0724603-4).

Conflict of Interest:

The authors declare no financial conflicts of interest.

Author Contribution

Xiuqi Jia and Xiang Li wrote the main manuscript text and Xinru Lv and Yi Li prepared figures 1-4. All authors reviewed the manuscript.

Data Availability

Raw metagenomic sequencing data have been deposited in the NCBI Sequence Read Archive under accession number SRR37633426,SRR37633428, SRR37633427,SRR37633425,SRR37633424,SRR37633423,SRR37633422,SRR37633420,SRR37633419,SRR37633421,SRR30213680,SRR36784985,SRR3674 Additional data and analysis scripts are available from the corresponding author upon reasonable request.

References

1. Essack S. Antibiotic resistance and One Health: a mapping project[J]. Lancet Global Health. 2018;6:S27.
2. Luo Y, Tan L, Zhang H, et al. Characteristics of wild bird resistomes and dissemination of antibiotic resistance genes in interconnected bird-habitat systems revealed by similarity of bla TEM polymorphic sequences[J]. Volume 56. Environmental Science & Technology; 2022. pp. 15084–95. 21.
3. Wang Y, Qian Y, Shi C et al. Seasonal Dynamics and Stability of Gut Antibiotic Resistance Genes in Plateau Pika (Ochotona curzoniae) and Plateau Zokor (Eospalax baileyi)[J]. J Hazard Mater, 2026: 141112.
4. Larsen J, Raisen CL, Ba X, et al. Emergence of methicillin resistance predates the clinical use of antibiotics[J]. Nature. 2022;602(7895):135–41.
5. Cross BJ, Partridge SR, Sheppard AE. Impacts of mobile genetic elements on antimicrobial resistance genes in gram-negative pathogens: Current insights and genomic approaches[J]. Microbiol Res, 2025: 128340.
6. Li LG, Xia Y, Zhang T. Co-occurrence of antibiotic and metal resistance genes revealed in complete genome collection[J]. ISME J. 2017;11(3):651–62.
7. Gillieatt BF, Coleman NV. Unravelling the mechanisms of antibiotic and heavy metal resistance co-selection in environmental bacteria[J]. FEMS Microbiol Rev. 2024;48(4):fuae017.
8. Amato KR, Yeoman CJ, Kent A, et al. Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes[J]. ISME J. 2013;7(7):1344–53.
9. Holt CC, van der Giezen M, Daniels CL, et al. Spatial and temporal axes impact ecology of the gut microbiome in juvenile European lobster (Homarus gammarus)[J]. ISME J. 2020;14(2):531–43.
10. Greene LK, Williams CV, Junge RE, et al. A role for gut microbiota in host niche differentiation[J]. ISME J. 2020;14(7):1675–87.
11. Yang J, Xu Z, Wan D, et al. Pollution characteristics of heavy metals, antibiotic and antibiotic resistance genes in the crested ibis and their habitat across different lifestyle and geography[J]. Environ Res. 2024;261:119701.
12. Xi J, Tao H, Zhang Z et al. Captive breeding of specialty animals represents an overlooked yet critical reservoir for spreading antibiotic resistance genes[J]. ISME J, 2026: wrag009.
13. Demian WL, Cormier O, Mossman K. Immunological features of bats: Resistance and tolerance to emerging Viruses [J]. Trends Immunol. 2024;45(3):198–210.
14. Muloi DM, Wee BA, McClean DMH, et al. Population genomics of Escherichia coli in Livestock-keeping households across a rapidly developing urban Landscape [J]. Nat Microbiol. 2022;7(4):581–9.
15. Gilliver MA, Bennett M, Begon M, et al. Antibiotic resistance found in wild Rodents [J]. Nature. 1999;401(6750):233–4.
16. Benavides JA, Salgado-Caxito M, Opazo-Capurro A, et al. ESBL-Producing Escherichia coli Carrying CTX-M Genes Circulating among Livestock, Dogs, and Wild Mammals in Small-Scale Farms of Central Chile [J]. Antibiotics. 2021;10(5):510.
17. Treskova M, Kuhlmann A, Freise F, et al. Occurrence of Antimicrobial Resistance in the Environment in Germany, Austria, and Switzerland: A Narrative Review of Existing Evidence [J]. Microorganisms. 2022;10(4):728.
18. Muloi DM, Wee BA, McClean DMH, et al. Population genomics of Escherichia coli in Livestock-keeping households across a rapidly developing urban Landscape [J]. Nat Microbiol. 2022;7(4):581–9.
19. FasoliS FE. And rearíG, SandríC, Dond iF, Is aniG. Noninvasives amplingmethod forur inalysisandurineprote inprofileincaptive giraffes. JournalofVeterinaryDiagnosticIn vestigation. 2021;33(1):25–34.
20. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34(17):i884–90. 10.1093/bioinformatics/bty560.
21. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754–60. 10.1093/bioinformatics/btp324.
22. Li D, Liu CM, Luo R, Sadakane K, Lam TW. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 2015;31(10):1674–6. 10.1093/bioinformatics/btv033.
23. Hyatt D, Chen GL, Locascio PF, et al. Prodigal: prokaryotic gene recognition and translation initiation site identification[J]. BMC Bioinformatics. 2010;11(1):119–119. 10.1186/1471-2105-11-119.
24. Noguchi H, Park J, Takagi T. MetaGene: prokaryotic gene finding from environmental genome shotgun sequences. Nucleic Acids Res. 2006;34(19):5623–30. 10.1093/nar/gkl723.
25. Fu L, Niu B, Zhu Z, Wu S, Li W. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics. 2012;28(23):3150–2. 10.1093/bioinformatics/bts565.
26. Li R, Li Y, Kristiansen K, Wang J. SOAP: short oligonucleotide alignment program. Bioinformatics. 2008;24(5):713–4. 10.1093/bioinformatics/btn025.
27. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods. 2015;12(1):59–60. 10.1038/nmeth.3176.

28. Kilroy C, Stephens T, Greenwood M, Wech J, Brown L, Matthews A, Patterson M, Patterson M. Improved predictability of peak periphyton in rivers using site-specific accrual periods and long-term water quality datasets. Sci Total Environ. 2020;736:139362.
29. Clarke KR, Somerfield PJ, Gorley RN. Clustering in non-parametric multivariate analyses. J Exp Mar Biol Ecol. 2016;483:147–55.

Figures

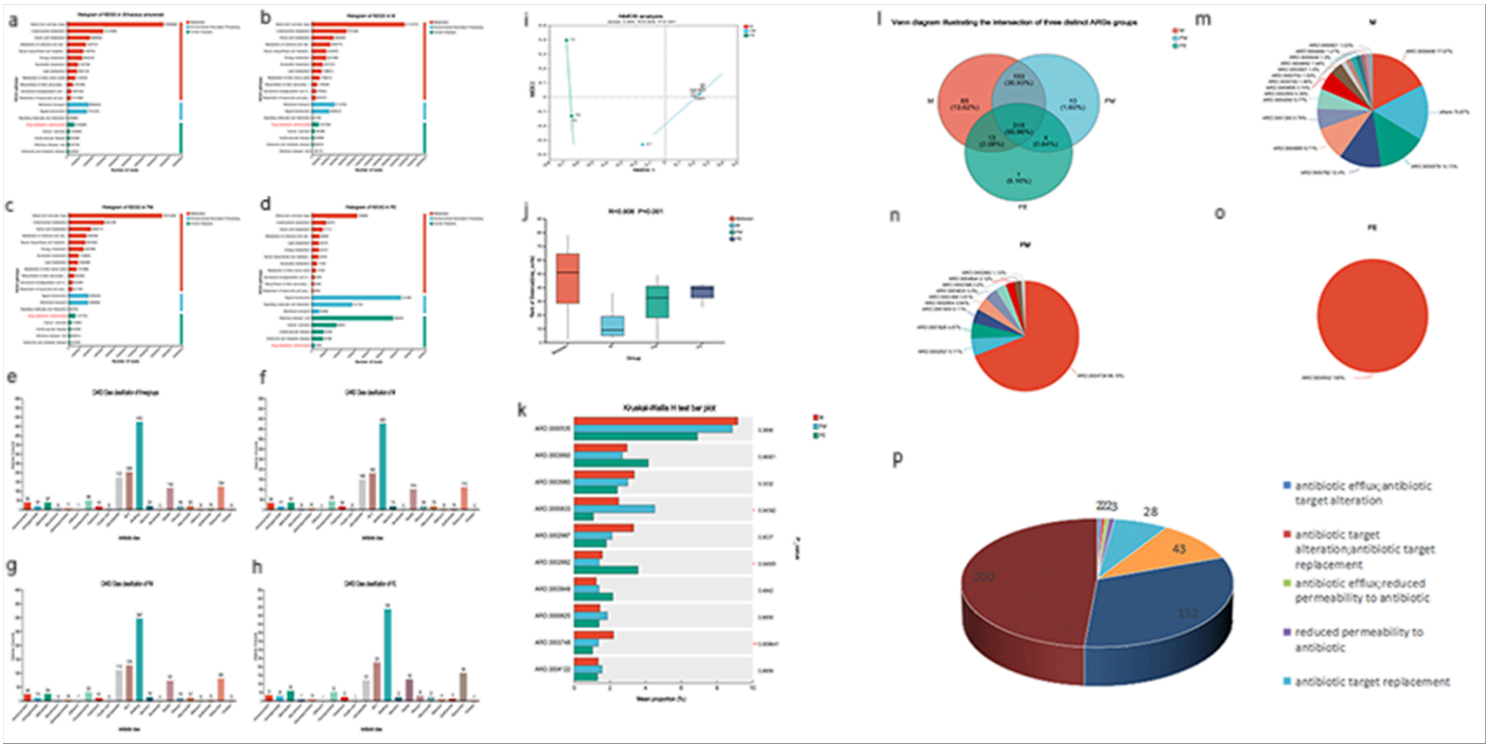


Figure 1

Developmentally regulated antimicrobial resistance gene profiles in the gut microbiota of *Erinaceus amurensis*. (a-d) The abundance of antimicrobial resistance-related sequences was substantially higher in adults compared to fetuses. (e-p) A total of 624 ARGs spanning multidrug, MLS, glycopeptide, and tetracycline classes were identified, with antibiotic efflux as the dominant resistance mechanism. Adults harbored more unique ARGs (l-o) and showed significant enrichment of genes such as evgS and lmrD (k), which correlated with the distinct clustering of adult and fetal samples in NMDS analysis (i-j, R = 0.608, P = 0.001).

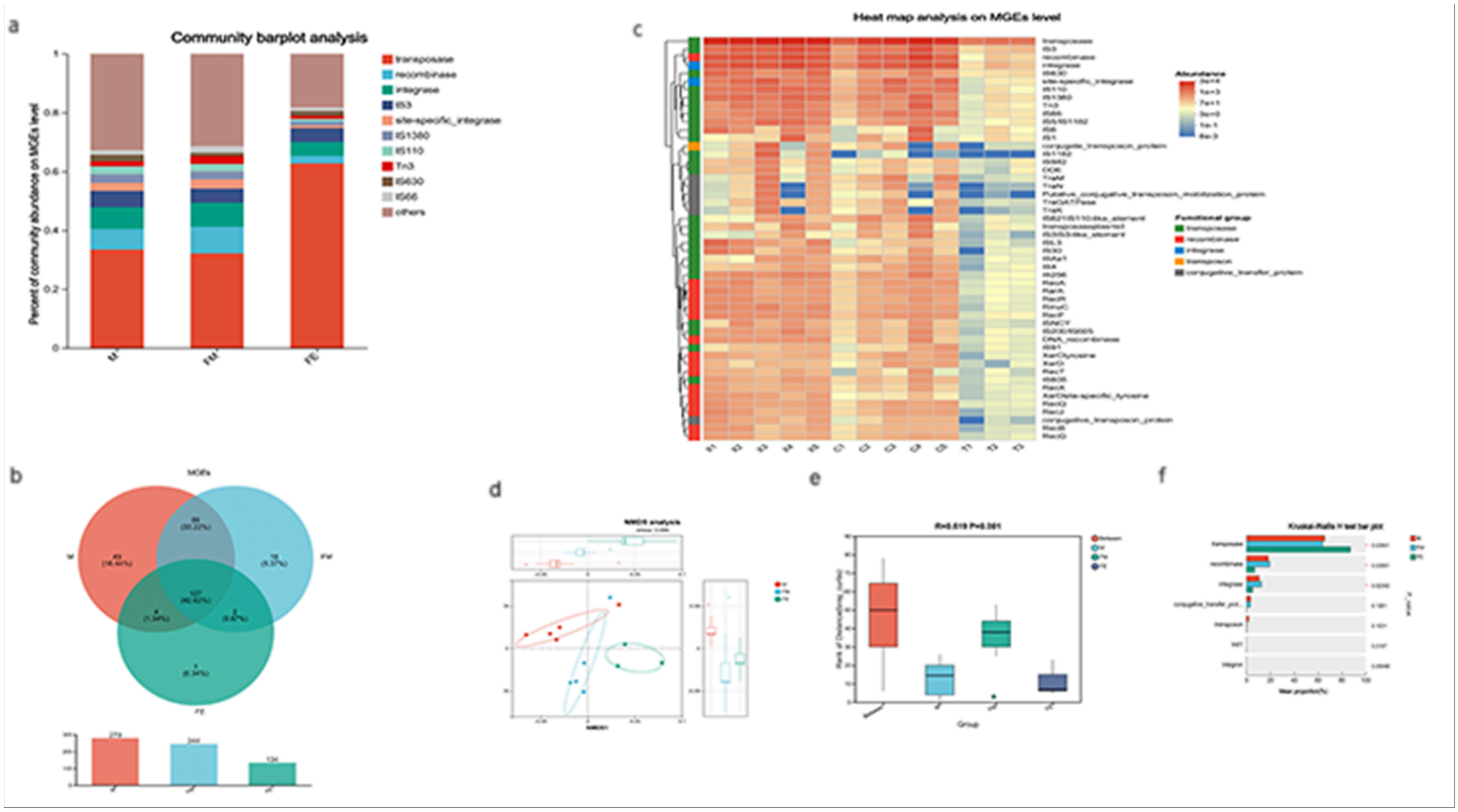


Figure 2

Landscape of mobile genetic elements (MGEs) in the gut microbiota across different life stages. (a) Abundance and composition of the most prevalent MGE classes, including transposases, recombinases, integrases, and specific insertion sequence (IS) elements. (b) Venn diagram showing the distribution and overlap of MGE subtypes among fetuses, adult females, and adult males. (c) Co-occurrence network analysis revealing strong positive correlations ($r > 0.6$) between multidrug resistance genes and specific MGEs, particularly integrases and IS3 elements. (d) Non-metric multidimensional scaling (NMDS) plot based on MGE profiles, showing distinct clustering by developmental stage (stress = 0.089). (e) Analysis of similarities (ANOSIM) confirming significant differences in MGE composition between groups ($R = 0.595$, $P = 0.001$). (f) Comparison of the relative abundances of key MGE groups (transposases, recombinases, integrases) across the three cohorts, with significant differences indicated ($P < 0.001$).

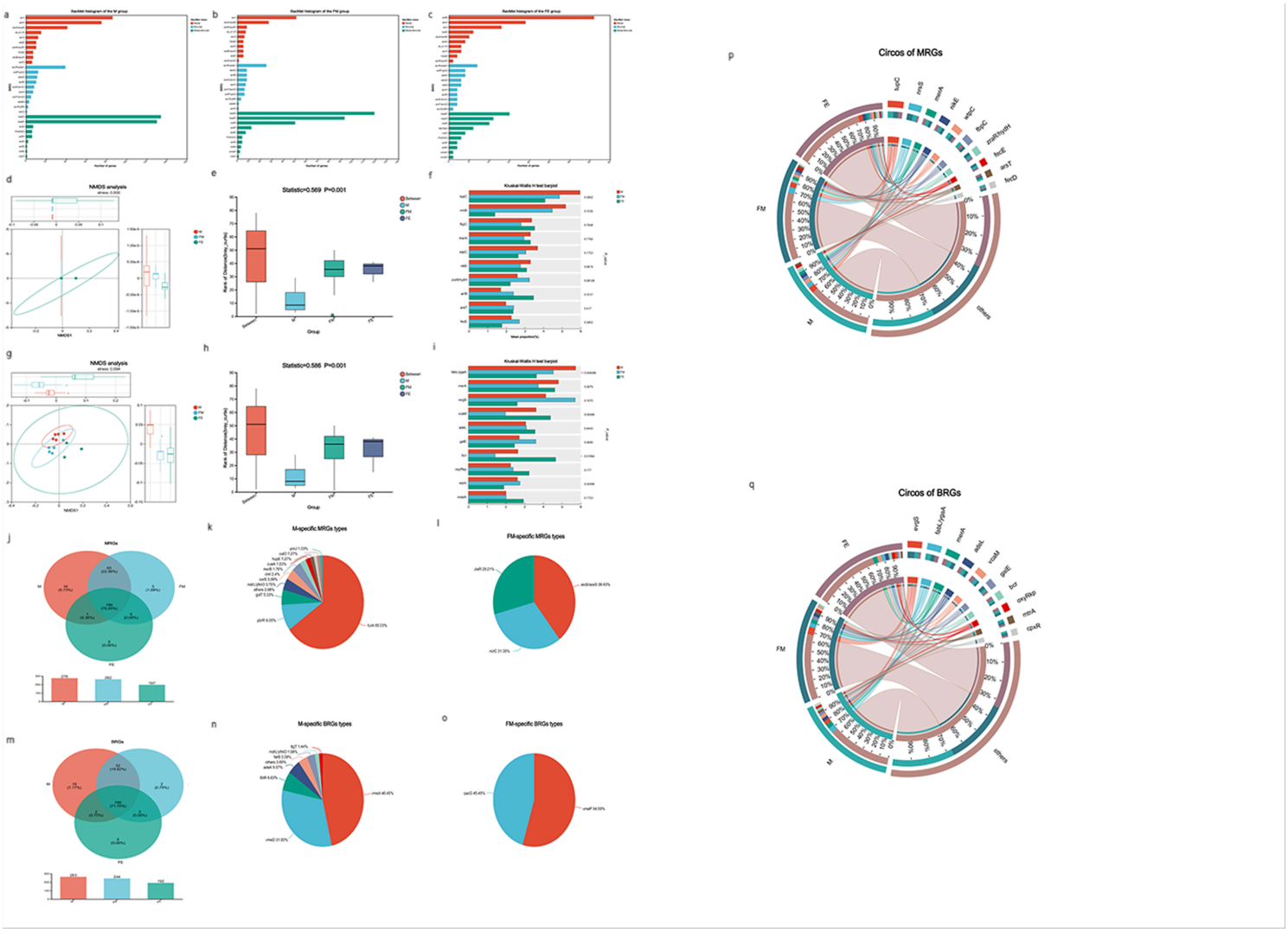


Figure 3

Developmental stage shapes the diversity and composition of metal and biocide resistance genes in the *Erinaceus amurensis* gut microbiota. (a-c) Abundance of BacMet-annotated metal resistance genes (MRGs) and biocide resistance genes (BRGs) followed a gradient of M > FM > FE. (d-h) NMDS and ANOSIM analyses confirmed significant compositional differentiation across developmental stages for both MRGs (R = 0.569, P = 0.001) and BRGs (R = 0.586, P = 0.001). Adult males harbored the highest diversity of MRGs (276 types) and unique BRGs (19 types) (f, m). (i) Genes involved in chemical stress response (merA, evgS, galE, oxyR-kp, mdeA) showed significant intergroup differences, with fabL/ygaA and bcr highly enriched in adults (P < 0.001). (j-l, p) Males were characterized by MRGs conferring resistance to multiple heavy metals (Fe, Pb, Cu, Zn, Cr, Hg, Ag, Ni), while females showed enrichment of Zn- and Ni-associated regulators (aioS/aoxS, ncrC, ziaR). (m-o, q) Male-specific BRGs included efflux pumps and transporters involved in resistance to surfactants and organic fungicides (vmeA, vmeD, adeA, farB, mdrL/yfmO, ttgT).

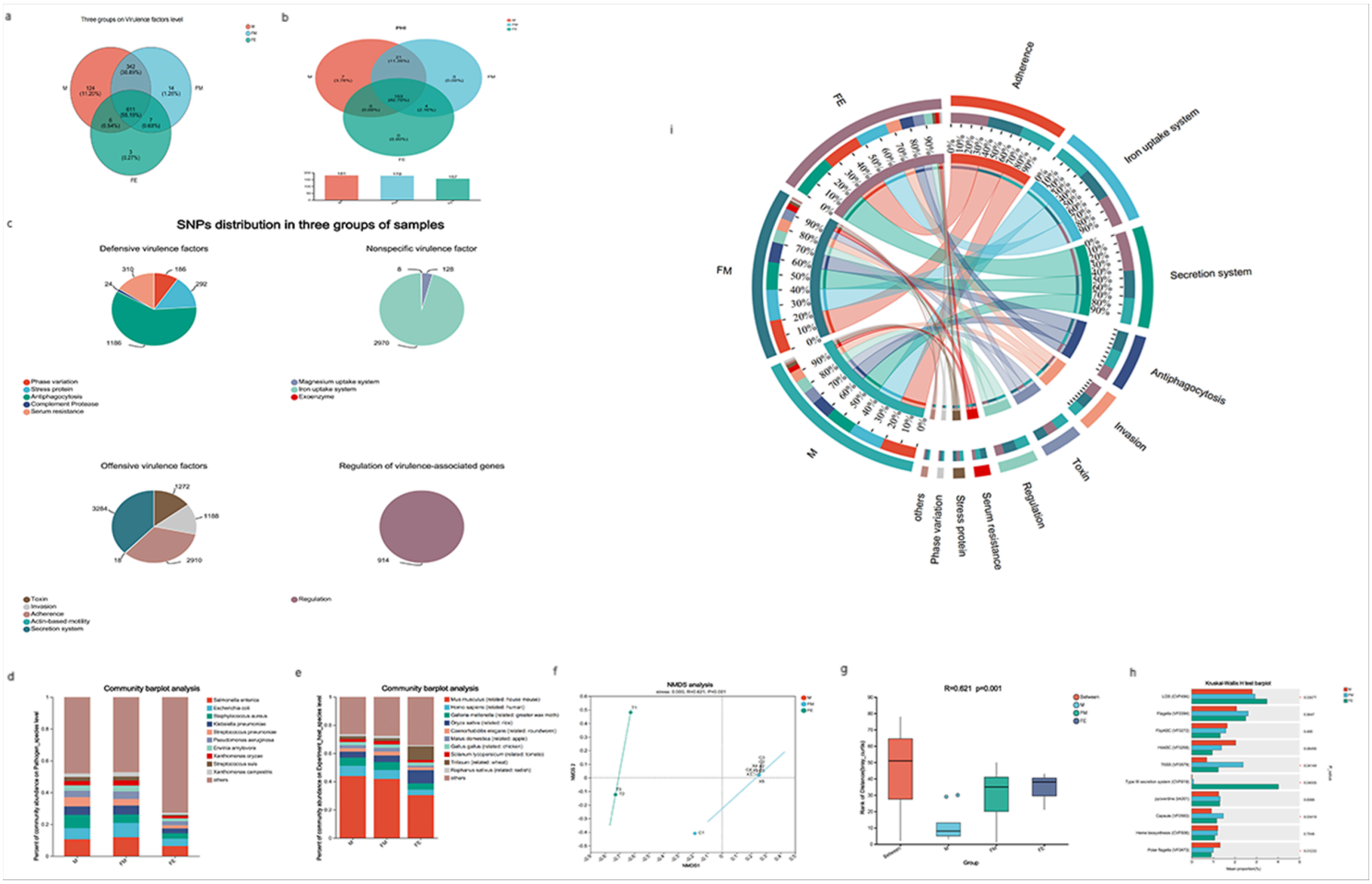


Figure 4

Developmental stage shapes the virulence factor landscape and pathogen-associated profiles of the Erinaceus amurensis gut microbiota. (a) VFDB annotation identified 506 virulence factors, with adult males, females, and fetuses harboring 124, 14, and 3 unique factors, respectively. (c) Major functional categories included antiphagocytosis, serum resistance, secretion systems, adherence, invasion, and toxins. (i) Fetal samples were enriched in secretion systems (42%) and adhesion (35%), whereas adults were dominated by defensive functions such as antiphagocytosis and serum resistance. (d-e) PHI-base annotation revealed 4,547 genes associated with major pathogens including Salmonella enterica, Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae, primarily targeting mammalian and poultry hosts. (f-g) NMDS and ANOSIM analyses confirmed significant compositional differentiation among developmental stages (R = 0.621, P = 0.001; stress < 0.05). (h) Stage-specific differences were observed in key virulence systems, including LOS (Haemophilus), T6SS (Shigella sonnei), polar flagella (Aeromonas hydrophila), capsule (Klebsiella pneumoniae), and T3SS (Yersinia), indicating functional divergence in host–pathogen interaction potential during development.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.jpg
- alltables.xlsx