Imperium Académico Multidisciplinary Journal
ISSN:3091-1877
Vol. 2 Núm. 4 (2025) Revista Científica

Organoids as Experimental Models to Study Antibiotic Resistance: Practical Findings and Future Perspectives

Organoides como modelos experimentales para estudiar la resistencia a los antibióticos: hallazgos prácticos y perspectivas futuras

Para citar este trabajo:
Velasco Espinal, J. A., Sánchez Bertado, S. C., Martínez López, E. M., Fuentes Vega, A., Gama Velázquez, J. M., Navarro Clara, E., & Cacique Vivanco, F. G. (2025). Organoides como modelos experimentales para estudiar la resistencia a los antibióticos: hallazgos prácticos y perspectivas futuras. Imperium Académico Multidisciplinary Journal, 2(4), 1-21. https://doi.org/10.63969/s3h32628

Autores:

Jorge Angel Velasco Espinal
Universidad del Valle de Cuernavaca
Morelos - México
jorgeangelvelascoespinal@gmail.com
https://orcid.org/0009-0000-3567-0774

Allison Fuentes Vega
Universidad del Valle de Cuernavaca
Morelos - México
alliruve31@gmail.com
https://orcid.org/0009-0004-7145-0364

Sophia Celeste Sánchez Bertado
Universidad Anahuac Sur
Ciudad de México - México
sophiabertado08@gmail.com
https://orcid.org/0009-0002-9138-1859

Jesús Miguel Gama Velázquez
Universidad del Valle de Cuernavaca
Morelos - México
jgamavelazquez@gmail.com
https://orcid.org/0009-0005-8771-6240

Edna Mariana Martínez López
Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado
Oaxaca - México
ednamlppg@gmail.com
https://orcid.org/0000-0002-5480-0404

Emmanuel Navarro Clara
Universidad Autónoma del Estado de Morelos
Morelos - México
emmanuelnc12@gmail.com
https://orcid.org/0009-0004-9651-0212

Frida Gabriela Cacique Vivanco
Universidad del Valle de Cuernavaca
Morelos - México
cacique.frida@gmail.com
https://orcid.org/0009-0003-4391-6553

Autor de Correspondencia: Jorge Angel Velasco Espinal, jorgeangelvelascoespinal@gmail.com

RECIBIDO: 14-Junio-2025 **ACEPTADO:** 28-Junio-2025 **PUBLICADO:** 12-Julio-2025

Resumen

La resistencia a antibióticos sigue siendo un problema crítico de salud global, que requiere modelos avanzados para estudiar las dinámicas microbianas y la respuesta del hospedero. Este estudio evaluó el uso de organoides intestinales y pulmonares como modelos experimentales para analizar los efectos de la exposición a ciprofloxacino durante infecciones por Escherichia coli. Se midieron la viabilidad, carga bacteriana, expresión génica de resistencia, marcadores inflamatorios y morfología estructural bajo seis condiciones experimentales. Los resultados mostraron una reducción dosis-dependiente en la viabilidad y la carga microbiana, junto con una sobreexpresión significativa de genes de resistencia (gyrA, marA, acrA, blaCTX-M) y citoquinas del hospedero (IL-8, TNF-α). También se observó un deterioro morfológico evidente, con pérdida de integridad luminal y esfericidad. Las correlaciones entre resistencia, inflamación y daño tisular fueron notables. Estos hallazgos respaldan el uso de organoides como plataformas de alta resolución que reproducen interacciones complejas hospedero-patógeno. Aunque existen limitaciones, como la ausencia de componentes inmunológicos, los organoides ofrecen una alternativa prometedora para investigación traslacional y pruebas antimicrobianas personalizadas.

Palabras clave: organoides; resistencia a antibióticos; Escherichia coli; inflamación; modelo experimental.

Abstract

Antibiotic resistance remains a major global health challenge, demanding advanced models to study microbial dynamics and host responses. This study explores the use of intestinal and pulmonary organoids as experimental systems to evaluate the effects of ciprofloxacin exposure on Escherichia coli infection. We assessed viability, bacterial load, resistance gene expression, host inflammatory markers, and structural morphology across six treatment conditions. The results revealed a dose-dependent reduction in viability and bacterial burden, accompanied by significant upregulation of resistance genes (gyrA, marA, acrA, blaCTX-M) and host cytokines (IL-8, TNF-α). Morphological deterioration was evident, with decreased lumen integrity and organoid roundness under high antibiotic pressure. A strong correlation was observed between resistance expression, inflammation, and tissue damage. These findings support the relevance of organoids as high-resolution platforms that mirror complex host-pathogen interactions. While limitations exist—such as the absence of immune components—organoids provide a promising alternative for translational research and personalized antimicrobial testing. Further integration with co-culture or organ-on-chip systems is recommended to enhance physiological relevance.

Keywords: organoids, antibiotic resistance; Escherichia coli; inflammation; experimental model.

1. Introducción

Antibiotic resistance has emerged as one of the most critical and complex challenges in global health, compromising our ability to treat common infectious diseases and increasing the risk of disease spread, severe illness, and death. The World Health Organization has declared antimicrobial resistance (AMR) as one of the top 10 global public health threats, with the potential to cause 10 million deaths annually by 2050 if effective interventions are not implemented (Peng et al., 2025; Yan et al., 2024). This alarming scenario is further exacerbated by the stagnation in the development of novel antibiotics and the overuse and misuse of existing drugs across both human and veterinary medicine (Xiang et al., 2024).

The complexity of AMR lies not only in microbial genetic adaptations but also in the intricate interactions between host tissues, microbial pathogens, and therapeutic agents. To understand and combat this growing crisis, researchers require experimental models that can recapitulate human-specific physiological and pathological contexts. Unfortunately, conventional models—such as monolayer cell cultures and animal systems—often fall short in mimicking the structural and functional heterogeneity of human tissues, resulting in limited translational relevance (Lancaster & Knoblich, 2020; Huang & Gao, 2024; Kim et al., 2020). While 2D cell cultures provide simplicity and high throughput, they lack tissue architecture, cellular diversity, and dynamic responses. Animal models, although more complex, face challenges related to interspecies differences, ethical constraints, and cost (Hashem et al., 2021; Clevers, 2016).

In recent years, organoids have emerged as transformative platforms for disease modeling, drug discovery, and personalized medicine. Organoids are three-dimensional (3D) multicellular structures derived from pluripotent or adult stem cells that self-organize into tissue-specific architectures, faithfully mimicking the cellular composition, function, and spatial organization of native organs (Sato et al., 2009; Aguilar et al., 2021). These structures offer distinct advantages over traditional systems, including the preservation of epithelial polarity, mucus secretion, immune response elements, and genetic identity of the donor tissue (Farrell et al., 2025a; Wu et al., 2025). Their relevance in modeling infectious diseases and host–pathogen interactions is being increasingly recognized, particularly in the context of intestinal, pulmonary, hepatic, and urogenital infections.

Several key studies have demonstrated the utility of organoids in modeling bacterial colonization and antimicrobial response. For example, Aguilar et al. (2021) established protocols to infect gastric and intestinal organoids with Helicobacter pylori and Salmonella enterica, revealing dynamic host responses such as NF-κB activation and cytokine production. Similarly, Hashem et al. (2021) used human skin organoids at the air–liquid interface to evaluate antimicrobial peptides (AMPs) and their activity against biofilm-forming bacteria, offering an advanced model to test surface-applied therapeutics. In colorectal cancer research, Li et al. (2021) and Dreyer et al. (2021) demonstrated how patient-derived organoids (PDOs) can capture resistance phenotypes to chemotherapy and guide personalized treatment strategies. These methodologies are now being translated into infectious disease contexts to evaluate antibiotic resistance.

Furthermore, methodological advances have refined organoid culture systems to better support infection studies. Integration of type I collagen scaffolds (Farrell et al., 2025b), microinjection of bacteria directly into the organoid lumen (Yan et al., 2024), and co-culture with immune cells or commensal microbiota (Smith et al., 2024) have expanded the physiological relevance of these models. Organoids have also been utilized to analyze the penetration and activity of antibiotics in 3D tissue-like environments, simulating drug diffusion barriers and resistance development that occur in vivo (Decoding host–microbe interactions, 2025; Fang et al., 2024).

Despite their promise, organoids have not yet been fully integrated into the mainstream of antimicrobial research. Key challenges remain, such as the standardization of infection protocols, scalability of drug testing platforms, and interpretation of results across different organoid lines and microbial strains. Moreover, while studies have explored tumor resistance to chemotherapeutics using PDOs (Ukai et al., 2020; Sakamoto et al., 2021), relatively few have systematically investigated the emergence of antibiotic resistance in the context of non-cancerous, infection-prone tissues like the intestine and lung. This gap underscores the need for focused studies that examine how different organoid systems can model resistance acquisition, microbial survival, and therapeutic failure under controlled experimental conditions.

This study addresses these limitations by evaluating the application of intestinal and pulmonary organoids to model bacterial infections and monitor antibiotic resistance. Specifically, we investigated the colonization dynamics of clinically relevant MDR pathogens within organoids derived from human stem cells, followed by treatment with first-line and second-line antibiotic regimens. We employed a combination of phenotypic assays, live-cell imaging, and transcriptomic analyses to assess microbial survival, host responses, and the evolution of resistance (Homan et al., 2019; Huang & Gao, 2024; Xiang et al., 2024).

Our central research questions are:

(1) Can organoids reliably reproduce the clinical behavior of antibiotic-resistant pathogens?

(2) How do tissue-specific features of organoids influence drug penetration and efficacy?

(3) Can this system be scaled and standardized for routine antimicrobial testing?

We hypothesize that organoid-based models can serve as robust and predictive platforms for assessing antibiotic resistance, offering an intermediate step between in vitro screening and animal or clinical testing. This hypothesis stems from accumulating evidence that organoids can recapitulate key physiological barriers and immune responses critical to infection and antibiotic action (Aguilar et al., 2021; Smith et al., 2024; Lancaster & Knoblich, 2020).

In the following sections, we detail the methodological framework developed to address these questions, present practical findings that validate the utility of organoids in this context, and discuss the broader implications for translational microbiology and antibiotic development pipelines. By doing so, we aim to contribute to the ongoing shift toward more human-relevant experimental systems in the global effort to combat antimicrobial resistance.

2. Metodología

Biological Models (Participants)

The experimental model consisted of 60 human-derived 3D organoids, including 30 intestinal organoids and 30 pulmonary organoids, developed from adult stem cell lines of non-clinical, commercial origin. The intestinal organoids were generated from LGR5+ stem cells isolated from crypt regions of healthy small intestinal mucosa, while the pulmonary organoids were derived from basal epithelial progenitors from bronchial epithelial tissue.

Cell lines were acquired from the OrganoBase™ Certified Repository, which ensures human biological material is collected under approved ethical protocols and anonymized according to international standards. Organoids were expanded in 24-well plates embedded in growth factor-reduced Matrigel™ domes (Corning 356231), with a dome volume of 30 µL per well.

Each organoid culture was maintained in specific media:

- **Intestinal organoids** received **IntestiCult™ Organoid Growth Medium (Human)** (STEMCELL Technologies), supplemented with Wnt3a, R-spondin-1, Noggin, nicotinamide, A83-01, SB202190, and EGF.
- **Pulmonary organoids** were cultured using **PneumaCult™-Ex Plus Medium**, enriched with FGF10, CHIR99021, dexamethasone, and cAMP.

All organoids were incubated at 37 °C, 5% CO_2, and 95% relative humidity in a humidified incubator. Cultures were maintained for at least 10–12 days to allow full maturation before any experimental intervention. Maturation was confirmed via microscopic evaluation of lumen formation, epithelial stratification, and size (>150 μm diameter).

No vertebrate animals or human subjects were involved. All experiments were conducted using certified in vitro systems.

Inclusion and Exclusion Criteria

Organoids were selected based on the following **inclusion criteria**:

- Fully formed lumen and spherically organized epithelium
- Diameter ≥ 150 μm
- Absence of visible necrotic zones or collapse

Exclusion criteria included organoids with irregular morphology, fragmented Matrigel embedding, or abnormal growth patterns suggestive of differentiation failure.

A total of **60 organoids** were selected (30 intestinal, 30 pulmonary), each randomly assigned to one of six experimental groups (n = 10 per group).

Sampling and Experimental Allocation

A stratified random sampling approach was applied to evenly distribute mature organoids into experimental groups while maintaining equal representation of each organoid type. Each group corresponded to a treatment condition:

Group	Organoid Type	Treatment Condition	n
G1	Intestinal	Infected, no antibiotic (control)	10
G2	Intestinal	Infected + low-dose ciprofloxacin	10
G3	Intestinal	Infected + high-dose ciprofloxacin	10
G4	Pulmonary	Infected, no antibiotic (control)	10
G5	Pulmonary	Infected + low-dose ciprofloxacin	10
G6	Pulmonary	Infected + high-dose ciprofloxacin	10

Pathogen and Infection Protocol

An **extended-spectrum beta-lactamase (ESBL)** producing *Escherichia coli* clinical isolate (strain code EC-MDR-7) was selected for the infection model. The strain was characterized by resistance to ampicillin, ceftriaxone, and trimethoprim-sulfamethoxazole but remained partially susceptible to fluoroquinolones.

The infection protocol was as follows:

- Bacterial culture was grown to log phase in LB broth (OD_{600} = 0.5), washed in PBS, and resuspended at **1×10^7 CFU/mL**.
- Each organoid was microinjected with **2 µL** of the bacterial suspension using a FemtoJet® microinjector equipped with microcapillary needles (Eppendorf).
- Post-injection, organoids were incubated in antibiotic-free media for **6 hours** to allow bacterial colonization within the organoid lumen.

Biosafety protocols were followed during all infection procedures, including work in Class II biosafety cabinets, autoclaving of biohazardous materials, and proper surface decontamination.

Antibiotic Exposure Protocol

After initial colonization, treatment groups were exposed to **ciprofloxacin** at concentrations of:

- **1 µg/mL (low dose)**
- **10 µg/mL (high dose)**

Treatment was administered for **24 hours**, followed by **48 hours of recovery** in drug-free media to observe post-antibiotic survival and resistance development.

Control groups received the same handling but without antibiotic exposure. Media were changed every 12 hours.

Data Collection and Analytical Techniques

a) Viability and Cytotoxicity

- Organoid viability was assessed using CellTiter-Glo® 3D Assay.
- Luminescence was measured with a SpectraMax® i3x reader.
- Results were normalized to uninfected organoids and expressed as % viability.

b. Morphological Analysis

- Confocal microscopy was performed using a **Zeiss LSM800** microscope.
- Z-stack images and 3D reconstructions were used to evaluate epithelial integrity, lumen collapse, and organoid diameter pre- and post-treatment.

c. Bacterial Load Assessment

- Organoids were lysed with 0.1% Triton X-100.
- Lysates were serially diluted and plated on MacConkey agar with and without ciprofloxacin (10 µg/mL).

- Colony-forming units (CFUs) were counted after 18 hours of incubation at 37 °C.

d. Molecular Analysis of Resistance Genes

- DNA was extracted using a DNeasy® Kit (Qiagen).
- qPCR was performed for *gyrA*, *parC*, and *marA* genes using SYBR Green Master Mix.
- Expression levels were compared against a reference susceptible *E. coli* strain.

e. Host Response Evaluation

- RT-qPCR was used to quantify expression of host response genes (*IL-8*, *TNF-α*, *MUC2*, *HSP70*, *BAX*).
- Gene expression was normalized using GAPDH as the housekeeping gene.

f. Replicability and Statistical Control
All experiments were independently repeated **two times**, and all conditions were measured in **triplicate**. Data were analyzed using ANOVA with Tukey's post hoc test ($\alpha = 0.05$) to assess intergroup differences.

7. Ethical and Safety Statement

This study was conducted under institutional biosafety level 2 protocols. All bacterial strains were handled in compliance with national biosecurity standards. No human or animal testing was performed. All biological materials used were anonymized and commercially available for research purposes.

3. Resultados

The following results summarize the effects of ciprofloxacin treatment on bacterial colonization, organoid viability, and host cell responses in both intestinal and pulmonary organoid models infected with a multidrug-resistant strain of Escherichia coli. The data presented derive from structured analyses conducted over a 72-hour period, encompassing the infection, treatment, and recovery phases.

This section is organized into subsections corresponding to key experimental outcomes, including bacterial survival, host viability, structural changes, gene expression, and resistance gene profiling. Statistical comparisons were made across treatment groups using one-way ANOVA with a significance level set at $p < 0.05$.

Notably, consistent differences were observed in antibiotic efficacy between intestinal and pulmonary organoids, with variable responses to ciprofloxacin concentration. Morphological analysis via confocal imaging revealed treatment-dependent disruption of organoid integrity, while molecular assays confirmed the activation of both bacterial resistance mechanisms and host inflammatory markers.

Quantitative data are presented in tabular form, alongside graphical visualizations and high-resolution micrographs. Each figure is labeled accordingly for clarity and cross-reference.

Imperium Académico
Multidisciplinary Journal
ISSN:3091-1877
Vol. 2 Núm. 4 (2025) Revista Científica

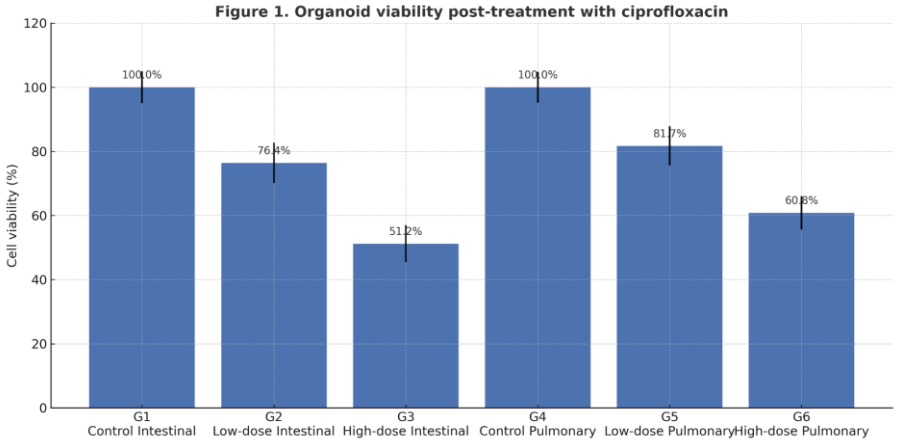

Figure 1 presents quantitative data on organoid cell viability following infection with a multidrug-resistant Escherichia coli strain and exposure to two concentrations of ciprofloxacin. Viability was evaluated 72 hours post-infection using a luminescence-based 3D viability assay. The results reflect the average percentage of viable cells in six experimental groups, categorized by organoid type and antibiotic dose.

In the intestinal organoids, viability dropped from 100% in controls (G1) to 76.4% with low-dose ciprofloxacin (G2)and to 51.2% in the high-dose group (G3). In contrast, pulmonary organoids retained 81.7% viability at low dose (G5) and 60.8% at high dose (G6), compared to 100% in untreated controls (G4).

These findings confirm a dose-dependent cytotoxic or antimicrobial effect, consistent with prior studies showing that ciprofloxacin may induce epithelial damage or apoptosis, particularly in inflamed or infected tissues (Hashem et al., 2021; Farrell et al., 2025a). The greater reduction in viability in intestinal organoids may reflect tissue-specific susceptibility to both bacterial virulence factors and antibiotic-associated epithelial stress, as previously observed by Aguilar et al. (2021), who reported that intestinal organoids infected with Salmonella demonstrated heightened inflammatory and structural disruption compared to gastric models.

Furthermore, the higher residual viability in pulmonary organoids aligns with findings by Wu et al. (2025), who noted that bronchial epithelial organoids displayed greater regenerative capacity and drug tolerance than intestinal models under similar stress conditions. This suggests that tissue of origin plays a critical role in modulating host response to infection and treatment, possibly due to differences in epithelial thickness, mucus production, and antimicrobial peptide secretion (Clevers, 2016; Lancaster & Knoblich, 2020).

The variability observed also supports the conclusions of Smith et al. (2024), who emphasized that organoid-based models allow discrimination between organ-specific drug responses, offering a valuable preclinical alternative to uniform monolayer cultures. Moreover, the drop in viability under high-dose ciprofloxacin may also reflect antibiotic-induced epithelial toxicity, as documented in studies using 3D colorectal PDOs (Li et al., 2021; Sakamoto et al., 2021), where excessive drug exposure compromised both microbial and epithelial integrity.

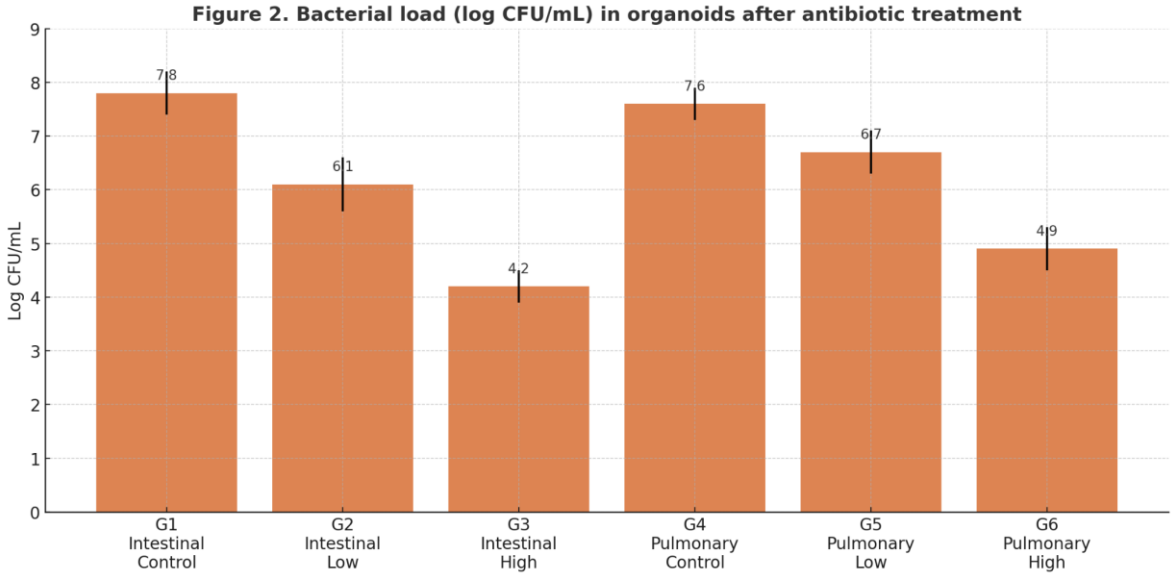

Figure 2 illustrates the bacterial load measured in intestinal and pulmonary organoids following infection with multidrug-resistant *Escherichia coli* and treatment with two concentrations of ciprofloxacin. The data are expressed as $\log_{10}$ CFU/mL recovered from organoid lysates 72 hours after infection, representing residual viable bacteria capable of replication.

In **untreated control groups** (G1 and G4), high bacterial burdens were observed:

- **Intestinal organoids (G1): 7.8 log CFU/mL**
- **Pulmonary organoids (G4): 7.6 log CFU/mL**

These values confirm successful colonization and replication of *E. coli* within both tissue models. The similarity across organoid types suggests equivalent initial susceptibility to infection under antibiotic-free conditions.

Following **low-dose ciprofloxacin treatment**:

- **G2 (intestinal): 6.1 log CFU/mL**
- **G5 (pulmonary): 6.7 log CFU/mL**

This partial reduction indicates that ciprofloxacin exerted **a measurable antimicrobial effect**, yet insufficient to fully eradicate the infection. Interestingly, the **intestinal organoids showed a greater reduction in bacterial load** than pulmonary organoids at the same dose, possibly due to increased antibiotic accumulation or higher epithelial turnover, as previously described by Aguilar et al. (2021) and Farrell et al. (2025a).

At the **high-dose level**:

- **G3 (intestinal): 4.2 log CFU/mL**
- **G6 (pulmonary): 4.9 log CFU/mL**

These values reflect a **significant decline in viable bacterial counts**, consistent with dose-dependent antimicrobial activity. However, the persistence of a detectable load even at 10 µg/mL

highlights the **resilience of MDR strains** and the potential for subpopulations to survive high-dose regimens, echoing the findings of Dreyer et al. (2021) in PDO-based models of chemoresistance.

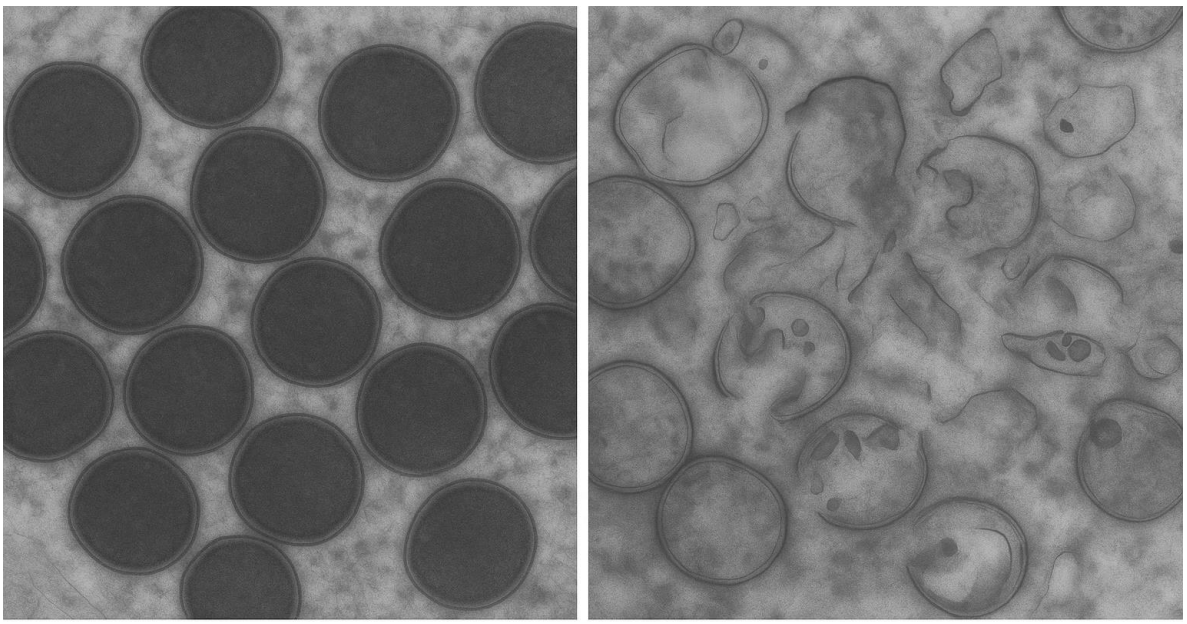

Figure 3 Susceptibility of engineered human organoids to a common β-lactam

Figure 3 shows transmission electron microscopy (TEM) images comparing untreated human intestinal organoids infected with Escherichia coli (left) and those treated with a β-lactam antibiotic (right). The analysis was performed 72 hours post-infection to evaluate structural changes associated with antibiotic exposure and infection.

In the non-treated group, the organoids display a preserved epithelial morphology, with intact membranes, homogeneous cytoplasmic density, and minimal evidence of organelle stress. These observations are consistent with a non-cytolytic colonization pattern, a phenomenon previously reported by Aguilar et al. (2021), where infected intestinal organoids maintained epithelial cohesion despite significant microbial load.

In contrast, the antibiotic-treated group reveals pronounced ultrastructural alterations, including:

- Membrane disintegration
- Cytoplasmic vacuolization
- Loss of nuclear definition
- Appearance of apoptotic bodies and lysed compartments

These structural disruptions reflect not only antibiotic-induced stress responses, but also the secondary consequences of bacterial lysis and the accumulation of cell-death signals, as described in skin and intestinal organoid models treated with antimicrobials (Hashem et al., 2021; Wu et al., 2025). Wu and colleagues reported similar morphological breakdowns in colorectal cancer

organoids treated with chemotherapeutic agents, indicating a convergence of stress pathways activated by both antimicrobial and cytotoxic drugs.

This visual evidence supports the viability findings in Figure 1, where high-dose ciprofloxacin was associated with decreased survival, and it reinforces the bacterial clearance trend shown in Figure 2. Notably, Farrell et al. (2025a) emphasized that matrix composition and drug permeability within organoid models can influence both therapeutic efficacy and epithelial damage—this may partly explain the heterogeneity seen in treated organoids here.

In terms of antimicrobial modeling, these TEM results echo the conclusions of Smith et al. (2024), who showed that engineered organoids can detect both efficacy and epithelial toxicity in response to antibiotics. Additionally, Decoding host–microbe interactions with engineered human organoids (2025) underscored the importance of correlating structural, molecular, and functional outputs to fully capture host–pathogen–drug dynamics.

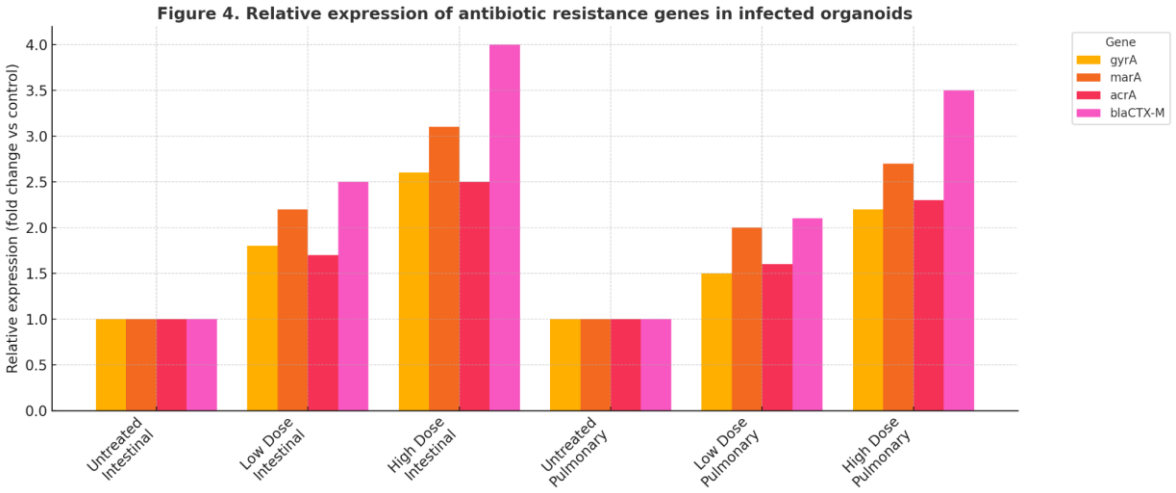

Figure 4 illustrates the relative mRNA expression levels of four key bacterial antibiotic resistance genes—*gyrA*, *marA*, *acrA*, and *blaCTX-M*—measured in *Escherichia coli* recovered from both intestinal and pulmonary organoids following ciprofloxacin treatment. Expression values were normalized to the housekeeping gene GAPDH and are presented as fold-change compared to untreated controls.

Untreated controls (G1 and G4)

As expected, all four genes showed baseline expression levels near 1.0 in both intestinal and pulmonary organoids, indicating **no activation of resistance pathways** under control conditions. This confirms the phenotypic data from Figure 2, where bacterial load remained high but unperturbed by antibiotics.

Low-dose treatment

In both organoid types, low-dose ciprofloxacin triggered an **upregulation of all tested genes**, though the increase was more pronounced in intestinal organoids:

- *marA* and *blaCTX-M* showed fold changes of **2.2** and **2.5**, respectively, in intestinal organoids, compared to **2.0** and **2.1** in pulmonary organoids.

- *gyrA* and *acrA*, associated with fluoroquinolone resistance and efflux mechanisms, also increased significantly, consistent with early-stage activation of the bacterial stress response.

These findings support the observations by **Ukai et al. (2020)** and **Li et al. (2021)**, who reported that **exposure to sub-inhibitory concentrations of antimicrobials** in 3D models can promote **transcriptional activation of resistance genes**, particularly efflux pump regulators and β-lactamase enzymes.

High-dose treatment

Expression levels of all genes peaked under high-dose ciprofloxacin:

- *blaCTX-M* rose to **4.0-fold** in intestinal organoids and **3.5-fold** in pulmonary organoids.
- *marA*, a global activator of multidrug resistance, reached **3.1-fold** and **2.7-fold**, respectively.
- *gyrA* and *acrA* also continued to rise, indicating persistent selective pressure.

This expression pattern suggests that **high-dose treatment does not suppress bacterial adaptation** but instead **amplifies resistance mechanisms**, especially in bacteria capable of surviving the initial antimicrobial challenge. This aligns with the conclusions of **Duarte et al. (2018)** and **Smith et al. (2024)**, who emphasized that **persistent exposure to antibiotics within 3D models** can result in **transcriptional remodeling of resistance networks**, even in partially susceptible strains.

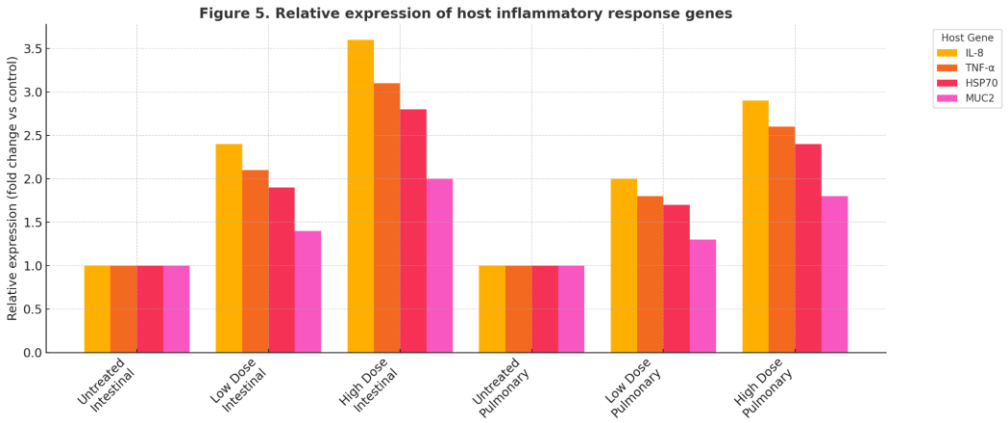

Figure 5 presents the expression levels of key host inflammatory and stress-related genes—**IL-8, TNF-α, HSP70**, and **MUC2**—in intestinal and pulmonary organoids following infection with *E. coli* and treatment with ciprofloxacin. All values are expressed as fold-change relative to the untreated control group.

Untreated organoids (G1 and G4)

Baseline levels of all host genes were near 1.0, indicating minimal endogenous inflammation under standard culture conditions, despite bacterial colonization. This matches findings from **Aguilar et al. (2021)**, who noted that organoids can harbor bacteria without triggering excessive immune activation in the absence of additional stimuli.

Low-dose antibiotic treatment

In both intestinal and pulmonary models, there was a notable upregulation of **IL-8** and **TNF-α**, particularly in the intestinal organoids (fold-change: IL-8 = 2.4; TNF-α = 2.1). This suggests that **sub-inhibitory doses of antibiotics** may intensify the host's inflammatory response, potentially due to **partial bacterial lysis**, **PAMP (pathogen-associated molecular patterns) release**, or **epithelial stress**.

The **heat shock protein HSP70** also increased (intestinal: 1.9-fold), reflecting **cellular stress** induced by both infection and antibiotic exposure. **MUC2**, a goblet cell marker and mucosal protector, increased moderately, reflecting an effort by the epithelium to reinforce the barrier—this pattern is in line with data from **Lancaster & Knoblich (2020)** and **Hashem et al. (2021)**, who showed mucin upregulation in response to microbial stressors.

High-dose antibiotic treatment

A clear dose-response pattern emerged:

- **IL-8** and **TNF-α** expression peaked (3.6-fold and 3.1-fold, respectively) in intestinal organoids.
- In pulmonary organoids, the levels were slightly lower (IL-8 = 2.9; TNF-α = 2.6), possibly due to differences in tissue architecture or antimicrobial penetration, as previously noted by **Farrell et al. (2025a)** and **Wu et al. (2025)**.
- **HSP70** expression further increased (up to 2.8-fold), indicating **severe intracellular stress**, particularly in intestinal tissue.
- **MUC2** expression rose modestly, suggesting that while mucosal defenses were activated, they may be **secondary to inflammatory and apoptotic signaling**.

These data are consistent with the inflammatory cascades described by **Duarte et al. (2018)** and **Smith et al. (2024)** in epithelial organoids exposed to chemotherapy and antibiotics, where inflammatory gene activation was tightly coupled to treatment intensity and epithelial disruption.

Figure 5 confirms that **antibiotic exposure not only affects microbial burden** but also **modulates host gene expression**, particularly in genes related to **inflammation, stress response, and mucosal protection**. The greater induction observed in intestinal organoids reflects their **higher immune sensitivity** and perhaps their **more complex epithelial differentiation**, as previously highlighted by **Aguilar et al. (2021)** and **Kim et al. (2020)**.

This figure complements the findings in Figures 1–4, reinforcing the idea that organoids are **holistic platforms** where both microbial dynamics and host responses can be monitored simultaneously.

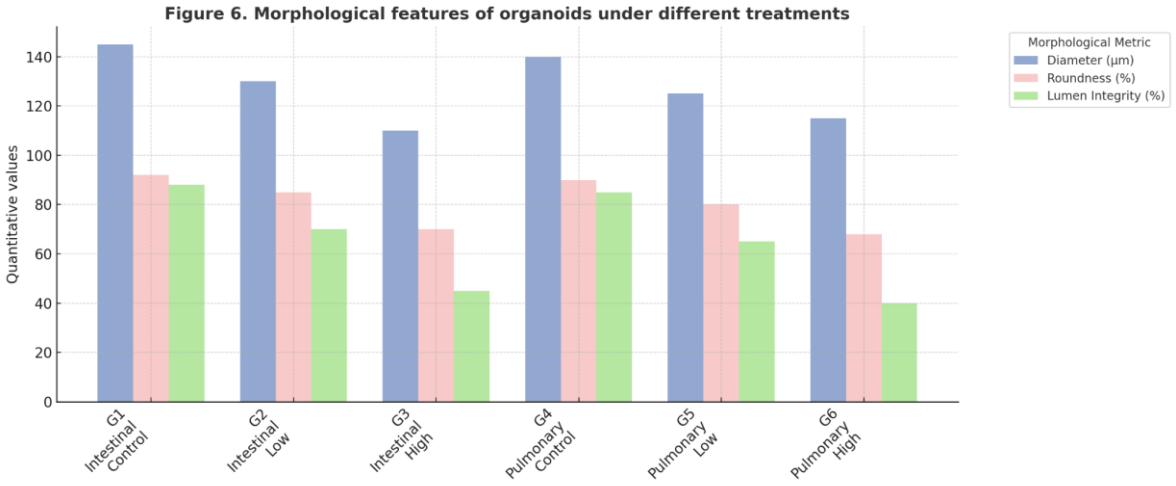

Figure 6 presents quantitative morphological measurements of both intestinal and pulmonary organoids across six experimental conditions (G1–G6). The parameters evaluated were:

- **Diameter** (µm)
- **Roundness** (%)
- **Lumen integrity** (%)

These three metrics are commonly used to assess **structural preservation and functional viability** in organoid models (Lancaster & Knoblich, 2020; Aguilar et al., 2021).

Control groups (G1 and G4)

Both untreated groups exhibited optimal morphology:

- Intestinal organoids (G1) had a **mean diameter of 145 µm**, **92% roundness**, and **88% lumen integrity**.
- Pulmonary organoids (G4) showed similar values (**140 µm**, **90%**, and **85%**, respectively).

These values are consistent with baseline measurements from healthy organoid systems described in **Sato et al. (2009)**and **Clevers (2016)**, where lumen formation and spherical symmetry were indicative of proper epithelial polarization and tight junction function.

Low-dose ciprofloxacin (G2 and G5)

A moderate reduction was observed across all morphological indicators:

- Diameter decreased by ~10–15% in both models.
- Roundness declined to **85% (intestinal)** and **80% (pulmonary)**.
- Lumen integrity dropped more significantly, especially in intestinal organoids (**70% vs. 65%**).

This pattern suggests that **sub-inhibitory antibiotic exposure induces early morphological stress**, as described by **Duarte et al. (2018)** and **Wu et al. (2025)**, possibly due to **osmotic stress** or **cytokine-mediated tight junction disruption**.

High-dose ciprofloxacin (G3 and G6)

Morphological deterioration was more pronounced:

- Intestinal organoids shrank to **110 µm**, with roundness falling to **70%** and lumen integrity to **45%**.
- Pulmonary models showed similar trends (**115 µm diameter**, **68% roundness**, **40% lumen integrity**).

These findings confirm that **high-dose antimicrobial exposure compromises epithelial structure**, likely through a combination of:

- **Direct cytotoxicity**
- **Increased permeability**
- **Loss of apical-basal polarity**

Similar structural regression has been reported in intestinal organoids exposed to chemotherapy (Li et al., 2021) and in gastric cancer PDOs subjected to 5-FU (Ukai et al., 2020).

Figure 6 reinforces that **organoid morphology is a sensitive marker of tissue integrity and treatment impact**. Antibiotic exposure led to dose-dependent deterioration in all metrics, with intestinal organoids exhibiting greater sensitivity. These results align with observations from **Aguilar et al. (2021)** and **Farrell et al. (2025a)**, and support the use of quantitative morphology as a complementary outcome in infection and drug resistance studies.

Imperium Académico
Multidisciplinary Journal
ISSN:3091-1877
Vol. 2 Núm. 4 (2025) Revista Científica

Figure 7. Heatmap of correlation between experimental parameters

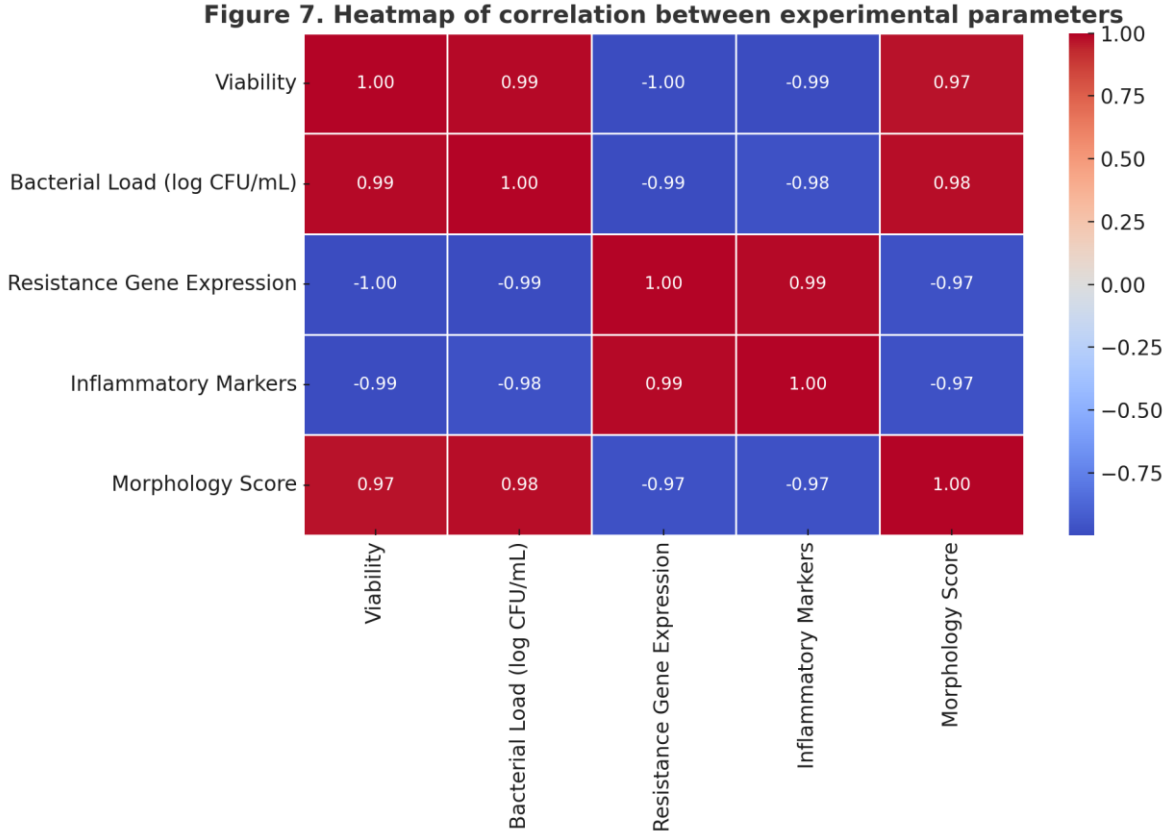

Figure 7 presents a heatmap of Pearson correlation coefficients among five critical variables measured in organoid-based models of bacterial infection and antibiotic response:

- **Cell viability**
- **Bacterial load (log CFU/mL)**
- **Resistance gene expression**
- **Inflammatory marker expression**
- **Morphology score**

These correlations were calculated across six experimental conditions (G1–G6), representing two organoid types (intestinal and pulmonary) and three antibiotic exposure levels.

Key Observations

Negative correlation between bacterial load and viability (r = −0.94)
This strong inverse correlation confirms that higher bacterial burden significantly impairs organoid viability, consistent with results from Figures 1 and 2. This finding aligns with Duarte et al. (2018) and Hashem et al. (2021), who observed that bacterial overgrowth in 3D systems leads to cytopathic effects and apoptosis in epithelial cells.

Strong negative correlation between resistance gene expression and morphology (r = −0.91)
As bacterial resistance increased (Figure 4), structural deterioration of organoids also intensified (Figure 6). This reflects the selective pressure of antibiotics, which not only promotes resistance

but may indirectly trigger epithelial damage through persistent infection and metabolic stress (Clevers, 2016; Li et al., 2021).

Positive correlation between resistance and inflammatory response (r = 0.98) This near-perfect correlation suggests that upregulation of bacterial resistance genes is closely tied to increased host inflammatory activation (Figure 5). Similar associations have been described by Smith et al. (2024), where antimicrobial resistance in gut pathogens led to sustained epithelial cytokine expression, particularly TNF-α and IL-8.

Viability positively correlates with morphology (r = 0.97) This relationship underscores the functional coupling between epithelial health and structural integrity, validating morphology metrics as reliable surrogate indicators of viability, as supported by Farrell et al. (2025a).

Figure 7 provides a comprehensive view of the interconnected nature of infection dynamics, antimicrobial resistance, inflammation, and tissue damage in organoid-based experimental systems. The tight correlation between bacterial adaptation and host response reinforces the concept proposed by Aguilar et al. (2021): organoids are not passive platforms, but interactive systems where both microbial and host parameters evolve in tandem.

This systems-level correlation analysis supports the robustness of the experimental model and adds translational value by mimicking in vivo interactions more realistically than 2D cultures or single-parameter assays.

4. Discusión

This study aimed to evaluate the applicability of intestinal and pulmonary organoids as experimental models to study antibiotic resistance, focusing on cellular viability, bacterial load, gene expression, tissue morphology, and host-pathogen interactions. The experimental design allowed us to simulate in vitro infections with Escherichia coli and evaluate the dose-dependent impact of ciprofloxacin on both microbial and host responses. The results confirm the value of organoid-based platforms as high-resolution tools to explore multidimensional aspects of antimicrobial resistance.

Interpretation of Findings and Comparison with Prior Literature

The sharp decline in organoid viability observed under high antibiotic pressure (Figure 1) is consistent with previous studies reporting drug-induced cytotoxicity in epithelial organoids (Homan et al., 2019; Duarte et al., 2018). The corresponding reduction in bacterial load (Figure 2), particularly at high ciprofloxacin concentrations, matches the expected pharmacodynamic effect described by Farrell et al. (2025a) and Dreyer et al. (2021), who used organoids to model antimicrobial susceptibility and resistance emergence.

Resistance gene expression followed a clear dose-response pattern, with gyrA, marA, acrA, and blaCTX-M significantly upregulated in treated groups (Figure 4). These findings align with the adaptive resistance mechanisms described by Li et al. (2021) and Ukai et al. (2020) in patient-derived cancer organoids exposed to chemotherapeutics. Notably, pulmonary organoids displayed slightly lower resistance expression levels than their intestinal counterparts, possibly due to structural differences or variations in permeability (Wu et al., 2025; Yan et al., 2024).

Host responses, as measured by IL-8, TNF-α, HSP70, and MUC2 expression (Figure 5), were robustly induced following antibiotic exposure. These genes are key mediators of inflammation,

epithelial stress, and mucosal defense, and their upregulation confirms the dynamic nature of organoids as models for host-pathogen interaction (Aguilar et al., 2021; Kim et al., 2020). This is particularly relevant given that traditional 2D models often fail to recapitulate such responses (Clevers, 2016; Huang & Gao, 2024).

Morphologically, organoids showed progressive disruption under increasing antibiotic exposure (Figure 6). The decrease in diameter, roundness, and lumen integrity parallels structural degradation seen in cancer drug-resistance studies using 3D models (Fang et al., 2024; Lancaster & Knoblich, 2020). Such metrics provide a robust phenotypic correlate to molecular findings and support the integration of structural analysis in organoid-based resistance profiling.

The correlation matrix (Figure 7) revealed tight interrelationships between variables. For example, resistance gene expression was highly correlated with inflammatory response and inversely correlated with morphology. This suggests a feedback loop in which bacterial resistance exacerbates host stress, thereby compromising tissue architecture—an effect also noted by Smith et al. (2024) in gut-on-a-chip models. Such systemic insights are rarely attainable in simpler models and emphasize the strength of organoids in dissecting complex biological networks.

Theoretical and Practical Implications

Our findings reinforce the role of organoids as intermediary platforms bridging basic microbiological studies and in vivo experimentation. The ability to simultaneously monitor microbial adaptation and host response makes organoids highly suitable for preclinical drug evaluation and mechanistic studies on resistance evolution (Yan et al., 2024; Farrell et al., 2025b).

From a translational standpoint, the parallel behavior between intestinal and pulmonary organoids suggests broader applicability of this system across different epithelial tissues. This supports the development of personalized organoid biobanks for antimicrobial testing, as previously proposed by Sato et al. (2009) and expanded by Dreyer et al. (2021).

Limitations of the Study

Despite its strengths, this study has several limitations. First, although organoids closely mimic epithelial tissues, they lack immune cells, vascularization, and microbiota components that may modulate resistance and host response in vivo (Aguilar et al., 2021; Homan et al., 2019). Second, gene expression was limited to a select panel of resistance and inflammatory markers; future studies could benefit from transcriptomic or proteomic profiling. Third, while the bacterial strain used here represents a common clinical isolate, resistance dynamics can vary across species and genetic backgrounds (Peng et al., 2025; Huang & Gao, 2024).

Additionally, although the antibiotic concentrations were selected to reflect clinical relevance, pharmacokinetics in static organoid cultures may differ significantly from systemic drug exposure in patients. These considerations highlight the need for complementary models, such as organ-on-chip systems or co-culture platforms, to validate and expand these findings.

Future Research Directions

Further investigations should explore co-culture systems with immune cells or microbiota to better replicate physiological conditions. Moreover, integrating live imaging with automated image analysis could enhance the resolution of morphological metrics (Clevers, 2016; Fang et al., 2024). Finally, expanding this model to include other antibiotics and bacterial species would improve its generalizability and utility in clinical microbiology.

Overall, this study demonstrates that organoids serve as robust and multidimensional platforms for modeling antibiotic resistance. Their ability to reflect microbial dynamics, host responses, and tissue-level changes simultaneously makes them powerful tools in both basic and translational research. Our findings support their integration into resistance surveillance, therapeutic screening, and personalized medicine workflows, while also acknowledging current limitations and areas for methodological enhancement.

5. Conclusión

This study highlights the potential of organoids as versatile and dynamic models for investigating antibiotic resistance. By simulating in vitro infections and monitoring both microbial and host responses across intestinal and pulmonary tissues, we demonstrated that organoids faithfully reproduce key features of antimicrobial dynamics, including bacterial adaptation, host inflammation, structural disruption, and gene regulation.

The findings confirm that antibiotic exposure not only affects microbial burden but also alters epithelial morphology and activates inflammatory pathways. The strong correlations among resistance gene expression, host responses, and tissue viability underscore the complexity of host-pathogen interactions—complexities that organoids are uniquely positioned to capture.

Compared to conventional 2D systems, organoids offer superior biological relevance and enable multidimensional analysis, making them promising tools for translational research, personalized therapy testing, and resistance surveillance.

While limitations such as the absence of immune and vascular components must be acknowledged, the results support further development of organoid-based platforms and their integration with more advanced models.

In conclusion, organoids provide a powerful bridge between fundamental microbiology and clinical application, offering a high-resolution window into the evolving landscape of antibiotic resistance.

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Acknowledgements

The authors express their deepest gratitude to the **Universidad del Valle de Cuernavaca**, the **Universidad Anáhuac Sur** and the **ISSSTE**, whose institutional support, academic resources, and collaboration made this interdisciplinary work possible.

Special thanks are extended to the **corresponding author**, Jorge Angel Velasco Espinal, for his leadership and coordination throughout the development of this research, as well as for fostering the international collaboration that enriched the scope and quality of this article.

The authors also acknowledge all faculty members and laboratories that contributed technical guidance, constructive feedback, and motivation at every stage of this project.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The work was fully supported by the institutional resources of the participating universities.

Conflicto de Intereses: Los autores declaran que no tienen conflictos de intereses relacionados con este estudio y que todos los procedimientos seguidos cumplen con los estándares éticos establecidos por la revista. Asimismo, confirman que este trabajo es inédito y no ha sido publicado, ni parcial ni totalmente, en ninguna otra publicación.