Review

The Emergence of New *E. coli* High-Risk Clones and Antibiotic Resistance: An Unstoppable Problem or Our Last Chance to Do Better?

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Review

The Emergence of New *E. coli* High-Risk Clones and Antibiotic Resistance: An Unstoppable Problem or Our Last Chance to Do Better?

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Abstract: Since the early 2000s, high-risk *E. coli* clones that presented a highly virulent and multidrug resistance profile have been identified, and their genome is completely sequenced. Molecular techniques make it possible to follow the evolution and spread of those clones in different parts of the world. The results of several studies monitoring the emergence of new high-risk *E. coli* clones showed an increase in the number of clones and the virulence strategies and in the emergence of clones with the ability to cause intestinal and urinary infections combined with a multidrug resistance profile. We certainly can't stop the emergence of new high-risk clones. The remaining questions are: How can we use the knowledge about *E. coli* high-risk clones to our advantage in the war against multidrug antibiotic resistance? How can we stop the spread of those clones and improve diagnostic and treatment stewardship? This is a challenge that requires collaboration and shared knowledge.

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Introduction

Bacteria, particularly *Escherichia coli* (*E. coli*), exhibit remarkable adaptability due to genomic plasticity and horizontal gene transfer. Horizontal gene transfer is the process by which bacteria can exchange genetic material with other organisms, enabling them to thrive in various environments and develop antibiotic resistance. *E. coli* is a well-studied bacterium, with strains categorized into intestinal and extraintestinal pathotypes. Extraintestinal pathogenic *E. coli* (ExPEC), especially uropathogenic *E. coli* (UPEC), is a significant cause of urinary tract infections (UTIs), often exhibiting multidrug resistance (MDR) due to the misuse of antibiotics [1,2].

The escalating threat of antimicrobial resistance (AMR), particularly the emergence of extended-spectrum β-lactamase (ESBL)-producing [3,4] Of enzymes, bestow resistance to β-lactam antibiotics, with CTX-M-15 being the most prevalent globally. The widespread dissemination of resistance genes, primarily via high-risk clones like ST131, further compounds the urgency of this issue.[5]

The use of antibiotics in agriculture, particularly in livestock, is a significant contributor to the transmission of resistant strains between animals and humans, with foodborne transmission playing a crucial role. Plasmids carrying resistance genes facilitate the spread of *E. coli* with ESBLs, including CTX-M enzymes, across clinical, community, and environmental settings. This underscores the importance of the work our audience is doing in implementing effective surveillance and control strategies to combat this critical issue.[6,7]

Extended-Spectrum β-Lactamase-Producing *E. coli*: Phylogenetic Groups, Clones, and Antimicrobial Resistance

Escherichia coli (*E. coli*) is a heterogeneous bacterium that predominantly lives in the gastrointestinal tract of humans, although its strains may lead to infections. Extraintestinal pathogenic *E. coli* (ExPEC) has become a severe threat, especially those capable of producing extended-spectrum β-lactamases (ESBL). Phylogenetic categorization of *E. coli* subgroups has also

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partially explained the virulence factors in the different strains. Infections are frequently connected with the B2 and D groups of strains; the A and B1 groups are commonly associated with the commensal microflora. Escaping high-risk clones in phylogenetic groups B2 and D with ESBL and fluoroquinolone resistance is a biomedical emergency that requires attention. [8,9].

Phylogenetic Groups and High-Risk Clones

One of the most dangerous clones is the HiRCC or high-risk clonal complex strains, represented in ST131, ST648, ST405, and others. Most research efforts are focused on the ST131 strains, which belong to the B2 phylogroup and have serotype O25. This concern about phylogenic clones has become widespread across the globe. ST131-associated infections include and are not limited to UTIs, IAIs, BSIs, and complex pneumonia, often of a moderate to severe nature. Notably, it is common in long-term care facilities, potentially affecting older adults; however, it was also found on pets and in food sources. [10–13].

Antibiotic Resistance and ST131

Research has proven the existence of TEM and CTX-M gene strains of *E. coli*, leading to the emergence of ESBL-producing *E. coli* organisms with fluoroquinolone-resistant types. The misuse of antibiotics such as cephalosporins and fluoroquinolones has created a selective pressure that has enabled the emergence of resistant strains of ST131. This antibiotic pressure has produced a high abundance of multidrug-resistant, or MDR, clones in outpatient and nursing home environments. However, much remains to learn, particularly about the ST131 type and its association with nosocomial outbreaks. The ST131 transmission dynamics remain a critical area for further research, highlighting the importance of ongoing efforts in this field. [1,14,15].

FimH30 and Fluoroquinolone Resistance

Fimbriae type 1, encoded by the *fimH* genes, is a critical factor in the virulence of ST131. The H30 variant of the *fimH* genes, which has gained prominence due to its association with fluoroquinolone resistance, is part of the fimH30 lineage. This lineage, identified through classical molecular typing techniques, shows homogeneity among the strains, suggesting a recent common ancestor. This connection to a shared evolutionary history is a fascinating aspect of our understanding of ST131. Most fimH30 ST131 strains are resistant to fluoroquinolone, with less than 1% resistant proportions of sensitive strains. Mutations in the *gyrA* and *parC* genes, which code for DNA gyrase and topoisomerase IV, are believed to cause this resistance. The fimH30 lineage has two main sublineages: the H30-R and H30-Rx. The H30-R sublineage is associated with fluoroquinolone resistance but does not produce ESBL, while the H30-Rx sublineage is fluoroquinolone-resistant and harbors $bla_{CTX\text{-}M15}$, a critical ESBL gene.[3,16].

Virulence Factors in ST131. The strains of ST131 can be grouped into four serotypes, A, B, C, and D, each with its unique set of virulence factors. These factors, including the A, especially the resistance genes such as $bla_{CTX\text{-}M15}$, contribute to the distinct characteristics of each serotype. Serotypes A and B, for instance, contain antibiotic-resistance genes, while serotype C is relatively expansive and more invasive than these two. Virotype D, on the other hand, is geographically restricted compared to the two but is also very virulent. The distribution of these diverse virulence factors among these biotypes, other than the fimH30 lineage, which appears progressive, adds to the complexity of our understanding of ST131.[17–19].

Resistance Mechanisms and Plasmid Transmission

The ST131 clone harbors various resistance determinants, including fluoroquinolone, aminoglycoside, and β-lactam antibiotic resistance. There are determinants spread by plasmid-mediated resistance, with the IncF family of plasmids being the most common ESBL gene vector type in ST131. IncF plasmids are carriers of multiple antibiotic resistance genes and nonresistance genes,

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such as iron metabolism and toxin production, which are virulence factors. These plasmids guarantee the survival and proliferation of the bacterium, even in the presence of antibiotics. [20–23].

Remarkably, the acquisition of resistance plasmids in ST131 is not a burden to fitness, as one might expect. There may be synergy between plasmids and chromosomal encoded factors that enhance the pathogenic potential of ST131. This finding underscores the urgent need to understand and potentially control the spread of antibiotic resistance. For example, ESBL-encoding plasmids have also been associated with increased biofilm formation and decreased motility, which may be useful in exploiting nutrients in specific environments. These insights could inspire the development of novel strategies for combating antibiotic resistance[3,24].

Global Dissemination of Resistance Genes

The global transmission of resistance genes among *E. coli* clones extends beyond ST131. The alarming rise of resistance genes, especially those leading to extended-spectrum beta-lactamases (ESBLs), is also linked to other significant clones like ST117 and ST38. These clones are frequently found in human and animal sources, such as poultry, underscoring a dangerous zoonotic connection in the spread of resistance genes. In Asia, we witness troubling pandemic levels of resistance in humans and livestock, driven by plasmids from the IncI1 and IncF families that function as critical vectors for transmitting *bla*$_{CTX-M}$ genes. For instance, blaCTX-M-55's spread in Brazil is directly associated with the ST131 clone.[3,25].

This clone likely acquired the resistance gene after its introduction to the country, emphasizing the urgent need to understand and combat the mechanisms behind the proliferation of antibiotic resistance. In conclusion, ExPEC is on the rise, and high-risk clones like ST131 are causing severe problems for global healthcare due to their virulence and antibiotic resistance. The evolution and spread of these strains, as revealed by phylogenetic classification, particularly groups B2 and D, are especially problematic. The ST131 clone, resistant to cephalosporins and fluoroquinolones, has become a significant cause of antibiotic-resistant infections, particularly in long-term care facilities and among the elderly. Despite intense antibiotic pressure, these clones have survived and increased due to a combination of virulence factors and plasmid-mediated resistance. However, there is hope. By improving our understanding of the mechanics of transmission and developing innovative treatment approaches, we can counteract the multidrug resistance of these high-risk clones and mitigate their global spread. [8,9,26,27].

Strategies of Diagnostic

The development of molecular diagnostic strategies has fundamentally transformed the management of infections caused by high-risk bacterial strains. These innovations enable personalized care, enhance epidemiological surveillance, and reduce unnecessary antibiotic use significantly. Whole genome sequencing (WGS) is an exact method for identifying and characterizing bacterial clones despite the challenges posed by high costs and the need for protocol validation. Next-generation sequencing (NGS), an advanced iteration of WGS, is indispensable for exploring microbial metagenomes and understanding mechanisms of pathogenicity and resistance. It is a critical tool for rapidly developing diagnostic tests for multidrug-resistant (MDR) infections, although its associated costs and complex data analysis must be acknowledged. [28–32].

Fourier transform infrared spectroscopy (FTIR) offers a rapid and cost-effective approach for bacterial identification through distinct "fingerprints" generated by infrared radiation interactions. Attenuated total reflectance FTIR (FTIR-ATR) further strengthens this method, making it an essential choice for routine laboratory implementation in identifying high-risk bacterial clones. Multilocus Sequence Typing (MLST) is crucial for tracking the relatedness of bacterial strains by sequencing conserved housekeeping genes. This method is vital for monitoring antimicrobial-resistant clones, particularly *E. coli* and *Klebsiella pneumonia*, with the Achtman scheme being the primary choice for *E. coli* identification.[33].

Pulsed-field gel electrophoresis (PFGE) effectively creates unique DNA fingerprints for bacterial strains, but it is time-consuming and technically demanding. Repetitive-sequence-based PCR Typing

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and Multilocus Variable-Number Tandem-Repeat Analysis (MLVA) also provide robust methods for distinguishing *E. coli* strains, each with distinct strengths. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a vital tool for rapid and precise microbial identification based on the mass-to-charge ratios of proteins and peptides, making it essential for the effective diagnosis and management of bacterial infections. In summary, these molecular techniques have revolutionized the landscape of bacterial identification and epidemiological tracking, establishing themselves as crucial resources for managing infections and combating antibiotic resistance. [34–37].

Alternatives Therapeutic Approaches

Due to the increasing antibiotic resistance of Extraintestinal Pathogenic *Escherichia coli* (ExPEC) strains and the adverse effects associated with traditional antibiotics, alternative therapeutic approaches are becoming essential for effectively combating bacterial infections. These methods aim to prevent or treat diseases, such as urinary tract infections (UTIs) caused by ExPEC, especially when standard treatments fail due to resistance. Vaccines are a promising tool for preventing infections, particularly those caused by uropathogenic *E. coli* (UPEC), a common cause of UTIs [34,38,39].

Several strategies are being explored:

1. Vaccines Based on Pili and Fimbrial Adhesins: Pili and fimbriae enable UPEC to adhere to host tissues, making them critical targets for vaccine development. Two primary approaches are pursued: - Purified Pili/Fimbriae-Based Vaccines: These vaccines leverage the immunogenic properties of pili or fimbrial fractions. For instance, vaccines based on Dr fimbriae have been shown to elicit a strong humoral immune response, producing antibodies that protect against mortality in UTI cases. However, they do not entirely prevent colonization in the bladder and kidneys. -Adhesin-Chaperone Complex-Based Vaccines: The FimC–FimH and PapD–PapG chaperone-adhesin complexes effectively block pathogen-host interactions and protect against UTIs. They stimulate antibodies that interfere with the function of FimH and PapG adhesins, which are critical for bacterial colonization. Current research focuses on optimizing FimC-FimH-based vaccines to enhance immune responses [40–42].

2. Vaccines Targeting Siderophores, Toxins, and Proteases: Siderophore-Based Vaccines: Siderophores are molecules that bacteria use to acquire iron, which is crucial for survival—especially in nutrient-deficient environments like the urinary tract. Vaccines targeting siderophore receptors (e.g., IroN, FyuA, IutA) have shown promise in animal models by generating humoral responses that inhibit the bacterial uptake of iron. - Toxoid and Protease-Based Vaccines: Toxins and proteases involved in UPEC pathogenesis, such as hemolysin (HlyA), are also targets for vaccination. Immunization with HlyA has been shown to reduce renal scarring in mice, although it does not entirely prevent bacterial colonization. These vaccines aim to neutralize toxins and proteases, thereby reducing their harmful effects [41–43].

Bacteriophage therapy is gaining renewed interest due to its potential for treating infections caused by antibiotic-resistant bacteria. Bacteriophages, viruses that specifically infect bacteria, provide a unique approach to eliminating bacterial pathogens. Advantages of Phage Therapy: Host Specificity: Unlike broad-spectrum antibiotics, bacteriophages target specific bacteria, preserving beneficial gut microbiota and minimizing side effects such as dysbiosis. - Co-evolution with Bacteria: Bacteriophages can co-evolve with bacteria, adapting to overcome resistance mechanisms. This dynamic interaction reduces the likelihood of developing phage resistance compared to conventional antibiotics[44–51].

Phage-Encoded Enzymes: Phages produce enzymes, such as endolysins and virion-associated peptidoglycan hydrolases (VAPGH), that degrade bacterial cell walls, leading to bacterial lysis. These enzymes effectively assist phages in destroying host bacteria.

Synergy with Antibiotics: In some instances, bacteriophage therapy can be combined with antibiotics to enhance treatment efficacy. For example, the lytic phage OMKO1 can restore antibiotic susceptibility in multidrug-resistant *Pseudomonas aeruginosa* by inducing evolutionary changes that

make the bacteria more sensitive to antibiotics. In summary, alternative therapeutic approaches like vaccines and bacteriophage therapy offer promising solutions to address the growing issue of antibiotic resistance in ExPEC infections. These strategies target bacterial virulence factors, toxins, and specific bacterial strains, providing more precise and less harmful treatment options.

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