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ORIGINAL RESEARCH

Rapid Plasmid-Mediated Acquisition of Erythromycin Resistance via *ermX* in *Corynebacterium striatum*: A 72-Hour Clinical Evolution

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Purpose: To investigate the genomic mechanisms driving rapid erythromycin resistance emergence in *Corynebacterium striatum* during clinical management of urinary tract infections.

Methods: Longitudinal analysis of four *C. striatum* isolates (C1-C4) from an ICU patient with candida coinfection was conducted using CLSI M45-A3-guided microbroth dilution and comparative whole-genome sequencing. Phylogenetic reconstruction (27 housekeeping genes) confirmed clonal origin, while CARD/PIPdb/ISfinder databases characterized resistome dynamics.

Results: Erythromycin susceptibility shifted from susceptible (minimum inhibitory concentration, MIC ≤ 0.25 μg/mL) to resistant (MIC = 4 μg/mL) within 72 hours, correlating with acquisition of a plasmid carrying *ermX* flanked by ISL3-family elements (ISCx1).

Conclusion: This first documentation of sub-72-hour *ermX* plasmid acquisition in *C. striatum* highlights critical infection control challenges in ICU settings. We recommend daily antimicrobial susceptibility testing (AST) profiling for *C. striatum* in ICU patients receiving macrolides, coupled with PCR screening for *ermX* to preempt resistance dissemination.

Keywords: *Corynebacterium striatum*, erythromycin, *ermX*, whole genome sequencing, resistance

Introduction

Corynebacterium striatum has been historically regarded as a component of the normal human skin and nasopharyngeal microbiota, with isolates frequently dismissed as contaminants in laboratory settings.[1,2] However, there has been a notable increase in the incidence of infections attributed to *C. striatum* in recent years, encompassing a spectrum of clinical presentations including endocarditis, meningitis, wound infections, urinary tract infections, bacteremia, and respiratory tract infections, with instances of hospital outbreaks.[2–8] Moreover, *C. striatum* is often associated with multidrug resistance, which poses a significant challenge to therapeutic management. Investigating the mechanisms of drug resistance in *C. striatum* is thus crucial for mitigating the spread of its resistance.

In the course of our clinical practice, we observed the rapid acquisition of resistance to erythromycin by a strain of *C. striatum* within a period of three days following the initiation of treatment. The objective of the present study is to elucidate the underlying resistance mechanisms of this strain through comparative whole-genome sequencing analysis conducted before and after the emergence of resistance. This will provide a theoretical framework to inform strategies for the prevention and control of drug resistance dissemination in *C. striatum*. We report the first documentation of plasmid-mediated *ermX* acquisition conferring erythromycin resistance in *C. striatum* within 72 hours, elucidating its molecular mechanism.

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Materials and Methods

Source of Bacterial Strains

Four *C. striatum* strains (C1-C4) were consecutively isolated from urine samples of an ICU patient over a 7-day period (September 7–14, 2020). The patient presented with a complicated urinary tract infection (UTI) complicated by co-infections with Candida albicans and *Corynebacterium striatum*. Antimicrobial therapy was administered sequentially, beginning with micafungin (for fungal coverage), followed by cefoperazone-sulbactam and meropenem (for broad-spectrum bacterial coverage). Four additional strains (C5-C8) from non-ICU patients during the same period served as phylogenetic controls. All isolates were preserved in 40% glycerol at −80°C prior to analysis. All samples were residual specimens obtained during routine hospital diagnostic procedures, not prospectively isolated for this specific research.

Methods

Strain Identification and Antimicrobial Susceptibility Testing

The isolated strains were identified using a MALDI-TOF mass spectrometer from Bruker, Germany, achieving a confidence score greater than 2.0. The minimum inhibitory concentration (MIC) of these strains to commonly used antibiotics was ascertained through the micro-broth dilution method, employing the Corynebacterium detection kit TDR CB-96 by Hunan Mindray Medical Technology Co., Ltd., in strict accordance with the manufacturer's protocols. Antimicrobial susceptibility testing followed CLSI M45-A3 (2020)[9] guidelines using cation-adjusted Mueller-Hinton broth microdilution. Quality control included Streptococcus pneumoniae ATCC 49619 and Enterococcus faecalis ATCC 29212.

Whole Genome Sequencing

Our study implemented a de novo assembly approach using *Corynebacterium striatum* ATCC 6940 as the reference strain. The isolated strain was whole-genome sequenced on Illumina NovaSeq 6000 (2×150 bp, average coverage 100×; effective sequencing depth ≥40× with 95% coverage at 20×) after DNA extraction and library preparation (NEBNext Ultra II FS kit). Raw reads were filtered (Q30≥85%) to remove low-quality bases (Phred≤20 over 40 bp), ambiguous bases (≥10 bp Ns), adapter sequences (≥10 bp overlap), host-derived reads (aligned to hg38), and PCR duplicates, retaining >90% of original data. De novo assembly was performed with SPAdes v3.15.4 (k-mer 21,33,55,77), yielding contigs (N50>100 kb) with 99% mapping rate.

Phylogenetic Tree Analysis Based on Housekeeping Genes

The genomes of the isolated strains were submitted to the Meiji Cloud tool (https://cloud.majorbio.com/page/tools.html) for housekeeping gene annotation, predicting the presence of genes including nusA, infC, rpsJ, rplC, rplB, rpsS, rpsC, rplP, rplN, rplE, rpoB, rplL, rpsB, frr, pgk, smpB, rplA, rplK, rpmA, pyrG, rplF, rpsE, rpsM, rpsK, rplM, rpsI, and rplS. The concatenated housekeeping gene sequences were subsequently analysed using MEGA11 software to deduce the phylogenetic relationships among the isolated strains.

Resistance Gene, Insertion Sequence, and Plasmid Analysis

Annotation of resistance genes was performed using the Comprehensive Antibiotic Resistance Database (CARD) in both strict and perfect modes (identity ≥90%, coverage ≥80%, E-value ≤1e-10). Insertion sequence analysis was performed using the ISfinder database (http://www-is.biotoul.fr/blast.php, 2023 update, E-value ≤0.001). Screening and analysis of plasmids and their components in the genomes of drug-resistant strains was facilitated by the Plasmids in Pathogens database (PIPdb).

Results

Erythromycin Resistance Correlates with ermX Acquisition

Phenotypic Analysis

The four clinical *C. striatum* strains (C1-C4) exhibited multidrug resistance to β-lactams (penicillin, ceftriaxone, meropenem), fluoroquinolones (ciprofloxacin), and clindamycin, while remaining susceptible to glycopeptides

Table 1 Key Antimicrobial Susceptibility Profiles

Strain	Erythromycin (μg/mL)	Clindamycin (μg/mL)	*ermX* Status
C1	<=0.25	>4	Negative
C2	4	>4	Positive
C3	4	>4	Positive
C4	4	>4	Positive

Table 2 Resistance Gene and Plasmid Features

Strain	Resistance Genes	Plasmid Features (Size/Location)	IS Elements/Location
C1	*Vant, vanW*	No	No
C2	*Vant, vanW, ermX*	10763bp/Scaffold29	ISC×1/Scaffold29
C3	*Vant, vanW, ermX*	10452bp/Scaffold31	ISC×1/Scaffold31
C4	*Vant, vanW, ermX*	*10161bp/Scaffold26*	*ISC×1/Scaffold26*

(vancomycin) and lipopeptides (daptomycin). Critically, a 512-fold increase in erythromycin MIC was observed in strains C2-C4 (MIC = 4 μg/mL) compared to the susceptible strain C1 (MIC ≤ 0.25 μg/mL) (Table 1). This abrupt resistance emergence coincided with *ermX* gene acquisition in C2-C4 (Table 2).

Genomic Basis of Erythromycin Resistance

Resistance Gene Profiling

Whole-genome sequencing revealed that *ermX*, a 23S rRNA methyltransferase gene conferring macrolide resistance, was exclusively present in resistant strains C2–C4 (Table 2). No alternative resistance mechanisms—including 23S rRNA mutations, efflux pumps (*mefA, msrD*), or other methyltransferase genes (*ermA, ermB*)—were detected (identity ≥90%, coverage ≥80%; CARD v3.2.1).

Mobile Genetic Elements

The *ermX* gene was located on a conserved 10–11 kb plasmid scaffold flanked by IS*L3* family transposases (Table 2). These plasmids shared 99% sequence identity across C2-C4, suggesting clonal dissemination. In contrast, the susceptible strain C1 lacked both *ermX* and IS*L3* elements, further supporting horizontal acquisition of *ermX* during ICU hospitalization.

Phylogenetic Analysis Confirms Clonal Spread

The housekeeping genes were analyzed on the online tool of Majorbio Cloud Platform (https://cloud.majorbio.com/page/tools/). The resultant phylogenetic tree analysis revealed that, among the strains under investigation, strains C1, C2, C3, and C4 shared the closest phylogenetic ties, suggesting a common ancestral origin. This finding indicates that the observed shifts in strain resistance are attributable to the acquisition of specific resistance mechanisms rather than being a consequence of recurrent infections by strains harbouring disparate resistance profiles (see Figure 1).

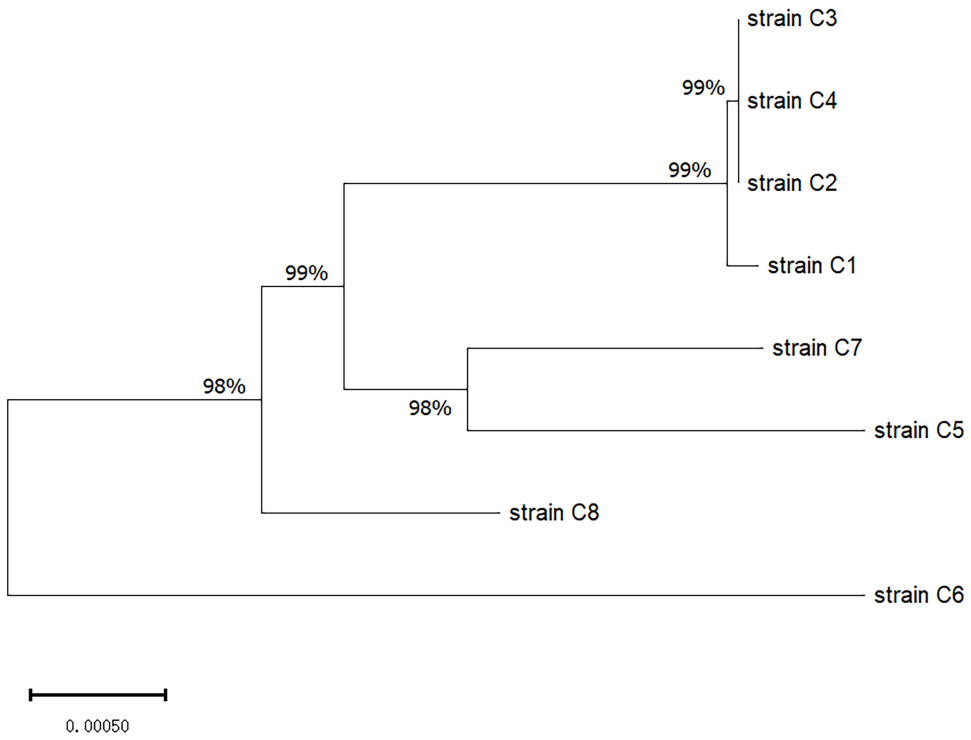

Figure 1 The phylogenetic tree constructed based on the sequences of 27 housekeeping genes. The tree was generated using the Neighbour-Joining (1,000 bootstraps) method as implemented in MEGA 11.0. Strains C5-C8 serve as background reference strains and are not included in the drug resistance analysis.

Discussion

In recent years, *Corynebacterium striatum* has emerged as a novel clinical pathogen that has garnered significant attention.[7,8,10–13] This interest is not only attributable to the diverse infections it can cause but also to its propensity for multidrug resistance, which presents a considerable challenge to clinical therapeutics.[8,14] Our study adds critical insights into this trend by documenting rapid in-host resistance evolution: a urinary isolate (C1) initially erythromycin-susceptible (MIC $\leq$ 0.25 μg/mL) acquired high-level resistance (MIC$\geq$ 4 μg/mL) within 72 hours, sustained across subsequent isolations (C2-C4). Core genome phylogeny confirmed clonal origin, excluding exogenous reinfection and implicating horizontal gene transfer (HGT) as the resistance driver.

The existing literature indicates that the primary mechanism of erythromycin resistance in *Corynebacterium striatum* is the presence of the *ermX* gene.[15–17] This gene mediates the methylation of ribosomal RNA, leading to alterations in the ribosomal structure, which in turn impedes the effective binding of MLS antibiotics (macrolides, lincomycin, and streptogramin) to the ribosomes, thereby diminishing their antibacterial efficacy.[15,18] A study to predict antimicrobial resistance using genomic sequences of 107 isolates of *Corynebacterium striatum* showed that the majority (97/107) of *Corynebacterium striatum* carry the *ermX* gene and *ermX* gave good agreement (83.33%) values with AST,[16] which is consistent with what we observed. The results of the CARD database analysis showed that the erythromycin-susceptible strains among our isolates carried the *vanT* and *vanW* genes, whereas the drug-resistant strains possessed additional *ermX* genes, confirming that the emergence of drug resistance is associated with the presence of *ermX* genes.

As is well known, the rapid dissemination of the *ermX* gene may be associated with a range of mobile genetic elements, encompassing insertion sequences, plasmids, and transposons.[19,20] In particular, the transposon Tn5432, which carries the *ermX* gene at the interspecies level, is essential for the mechanism of acquired resistance to erythromycin and clindamycin.[17,21,22] The results of plasmid annotation showed that the genomes of the erythromycin-resistant strains among our isolates contained a plasmid carrying the *ermX* gene, which was absent in the susceptible strains. Similarly, insertion sequence analysis showed that the *ermX* gene of the erythromycin-resistant strain annotated with four insertion sequences—ISC×1, IS1386, IS31831 and IS1207—which were not detected in the whole genome of the erythromycin-sensitive strain. Tn5432 is a composite transposon that carries a short insertion sequence, ISCx1, referred to as

a "genome scar".[19,23] Several studies have confirmed that the erm (X) gene often appears together with the ISCx1 element in the Tn5432 transposon.[22,24] We can therefore speculate that the plasmid carrying the *ermX* gene is the reason why our strain has acquired erythromycin resistance.

It is widely recognised that bacterial resistance to certain antimicrobial agents is often associated with their overuse. In this study, the strains under investigation were isolated from patients with urinary tract infections who had concomitant Candida albicans infections. The antimicrobials administered in these cases were micafungin and cefoperazone-sulbactam rather than macrolides. Although no macrolides were administered, β-lactam-induced SOS responses may have upregulated the plasmid conjugation machinery, as reported in Enterococcus.[25]

While our data robustly link *ermX*-bearing plasmids to resistance emergence, two paradoxes warrant attention: Accelerated HGT without selective pressure: Unlike Bifidobacterium, where DMSO/vorinostat enhance *ermX* transfer,[26] our patient received no such agents. This implies ICU-specific factors (eg, biofilm-rich catheters or phage-mediated transduction) may drive plasmid spread. Vancomycin susceptibility despite *vanT*/*vanW*: The vancomycin-susceptible phenotype (MIC ≤0.5 μg/mL) contrasts with *vanT*/*vanW* detection, suggesting non-functional alleles or regulatory suppression—a phenomenon needing transcriptomic validation.

The 72-hour emergence of ermX-mediated resistance necessitates a reevaluation of AST protocols for *C. striatum* infections. Our findings align with Wang et al showing ICU transmission clusters,[22] and Urrutia et al demonstrating ISL3-mediated plasmid transfer.[17] For patients infected with *C. striatum*, we recommend enhanced antimicrobial susceptibility test through more frequent AST intervals and preemptive ermX screening prior to macrolide therapy initiation.

This study has several important limitations that should be considered when interpreting the results. First, the single-patient case design limits the generalizability of the observed *C. striatum* resistance dynamics to broader clinical populations. Second, while genomic evidence strongly supports plasmid-mediated ermX transfer, the lack of experimental validation through conjugation assays or transcriptomic analysis of ermX expression represents a key methodological constraint. Future studies should address these limitations through: (1) prospective, multicenter surveillance of ermX acquisition rates in *C. striatum*, stratified by ICU antibiotic usage patterns and patient comorbidities; (2) functional characterization of ISL3-mediated plasmid transfer using in vitro biofilm models and murine infection assays.

Conclusions

In conclusion, our study documents the rapid emergence of erythromycin resistance in *Corynebacterium striatum* within a period of only three days. Genomic sequencing analysis revealed that this resistance shift was due to the acquisition of a plasmid containing the *ermX* gene. This discovery highlights the ability of *Corynebacterium striatum* to rapidly evolve resistance to erythromycin. However, the underlying cause remains elusive and requires further in-depth investigation. At the same time, it is vital to remain vigilant on the issue of bacterial resistance. Understanding the mechanisms of resistance is essential to inform strategies aimed at reducing the spread of antimicrobial resistance.

Ethical Statement

This study was reviewed and approved by the Ethics Committee of Deyang People's Hospital. The specimens used were anonymous residual samples collected during routine hospital procedures, with all identifiers permanently removed. This research posed no additional risks to patients and qualified for exemption from informed consent under retrospective study criteria.

Data Availability

The assembled genome files have been archived in the National Microbiology Data Center (NMDC) under the accession numbers NMDC60200991, NMDC60200992, NMDC60200993, and NMDC60200994.

Author Contributions

All authors made a significant contribution to the work reported, whether in conception, study design, execution, data acquisition, analysis and interpretation, or all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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