

RESEARCH ARTICLE

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Antibiotic Susceptibility Patterns and Genomic Characterization of *Cutibacterium acnes* Isolates from Healthy Individuals and Acne Patients

Xuong Mau Tu^{1,2} , Phong Xuan Huynh¹ , Lieu Thuy Thi Nguyen³ ,
Long Dang Hoang Bui¹ , Thanh Ngoc Nguyen¹  and Dung Ngoc Tran^{4*} 

¹Institute of Food and Biotechnology, Can Tho University, Can Tho City, Vietnam.

²Can Tho Hospital of Dermato-Venereology, Can Tho City, Vietnam.

³Faculty of Medicine, Tra Vinh University, Vinh Long Province, Vietnam.

⁴Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho City, Vietnam.

Abstract

Cutibacterium acnes is a dominant member of the human skin microbiota and is closely associated with the pathogenesis of acne vulgaris. The increasing emergence of antibiotic-resistant *C. acnes* strains has raised concerns regarding the long-term effectiveness of current therapies. This study aimed to characterize antibiotic susceptibility patterns and genomic features of *C. acnes* isolates obtained from healthy individuals and acne patients with different disease severities. A total of 45 participants were enrolled, including healthy controls and patients with mild and moderate-severe acne. *C. acnes* isolates recovered from pilosebaceous follicles were subjected to antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method against five antibiotics including clindamycin, tetracycline, azithromycin, trimethoprim-sulfamethoxazole (TMP-SMX), and doxycycline. Representative isolates were selected for whole-genome sequencing, followed by multi-locus sequence typing (MLST) and antimicrobial resistance gene annotation. The results showed that most isolates remained highly susceptible to tetracycline-class antibiotics and TMP-SMX, whereas resistance to macrolides and clindamycin was detected, particularly among moderate-severe acne isolates. Genomic analysis revealed notable genetic diversity among the isolates and identified a single antimicrobial resistance gene, *erm*(51), consistent with the observed resistance phenotype. Overall, antimicrobial resistance in the analyzed *C. acnes* population was limited but detectable. The integration of phenotypic susceptibility testing with whole-genome analysis provides valuable insights for antimicrobial resistance surveillance and supports rational antibiotic use in acne management.

Keywords: *Cutibacterium acnes*, Acne Vulgaris, Antibiotic Susceptibility, Antimicrobial Resistance, Whole-genome Sequencing, MLST

*Correspondence: tranngocdung@ctump.edu.vn

Citation: Tu XM, Huynh PX, Nguyen LTT, Bui LDH, Nguyen TN, Tran DN. Antibiotic Susceptibility Patterns and Genomic Characterization of *Cutibacterium acnes* Isolates from Healthy Individuals and Acne Patients. *J Pure Appl Microbiol.* Published online 01 August 2026. doi: 10.22207/JPAM.20.3.14

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INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit that affects a large proportion of adolescents and young adults worldwide. Its pathogenesis involves sebum overproduction, follicular hyperkeratinization, inflammation, and skin microbiota dysbiosis.¹ Among skin-resident microorganisms, *C. acnes* plays a central role, acting as a physiological commensal, while also contributing to inflammation under dysbiosis conditions.^{2,3}

Cutibacterium acnes is a Gram-positive anaerobic bacterium that preferentially colonizes sebaceous follicles due to its ability to metabolize sebum-derived lipids. Recent evidence indicates that acne severity is more closely associated with the genetic diversity and strain composition of *C. acnes* rather than its absolute abundance.⁴ Specific sequence types and phylotypes, particularly those belonging to phylotype IA1, have been strongly associated with inflammatory acne, whereas other lineages are more frequently detected on healthy skin.^{3,5}

Antibiotics, especially macrolides, lincosamides, and tetracyclines, have been widely used in acne treatment for decades. However, prolonged and sometimes inappropriate antibiotic use has contributed to the increasing emergence of antibiotic-resistant *C. acnes* strains, leading to reduced treatment efficacy and growing public health concerns.¹ Recent epidemiological studies have reported high resistance rates to macrolides and clindamycin, with substantial geographic variation and an overall increasing global trend.⁵⁻⁷

In parallel with phenotypic data, genomic studies have shown that antimicrobial resistance in *C. acnes* may arise through target gene mutations or acquisition of resistance genes mediated by mobile genetic elements.^{8,9} Nevertheless, integrated studies combining antibiotic susceptibility testing with whole-genome characterization of *C. acnes* isolates from different clinical groups remain limited, particularly in developing countries.^{2,5} Therefore, the present study aimed to evaluate the antibiotic susceptibility profiles of *C. acnes* isolates from healthy individuals and acne patients with varying disease severities and to characterize representative isolates using whole-genome sequencing and multi-locus sequence typing.

MATERIALS AND METHODS

Antibiotic susceptibility testing of *Cutibacterium acnes*

The study included 45 participants, comprising 30 patients with acne vulgaris and 15 healthy controls, stratified into healthy (n = 15), mild acne (n = 15), and moderate-severe acne (n = 15) groups. *Cutibacterium acnes* isolates obtained from pilosebaceous follicles were evaluated for antibiotic susceptibility using the Kirby-Bauer disk diffusion method with five antibiotics including clindamycin, tetracycline, azithromycin, TMP-SMX, and doxycycline. The experiment consisted of 45 isolates, including one reference strain and 44 clinical isolates, each performed in triplicate. Isolates were cultured in Fluid Thioglycolate Medium for 5 days, inoculated onto Mueller–Hinton agar, and incubated anaerobically at 37 °C for 5 days. Inhibition zone diameters were measured and interpreted as susceptible (S) or resistant (R) according to EUCAST breakpoints for *Staphylococcus* spp. and CLSI (2024) guidelines, as EUCAST species-specific criteria for *C. acnes* are currently unavailable. Based on the antibiotic susceptibility results, one *C. acnes* isolate exhibiting the highest level of antibiotic resistance was selected from each study group. In addition, one isolate from the healthy control group that was fully susceptible to all tested antibiotics was included as a comparator. These representative isolates were subjected to whole-genome sequencing to investigate the presence and genetic basis of antimicrobial resistance determinants.

Data processing and quality control

Raw sequencing data were initially assessed for quality using fastQC to examine key parameters, including per-base quality score distribution, GC content, adapter contamination, and sequence duplication levels.¹⁰ Following preliminary evaluation, reads were cleaned and optimized using fastQ by trimming low-quality regions, removing adapter sequences, and filtering out substandard reads to ensure high reliability for downstream analyses.¹¹

Genome assembly and quality assessment

Quality-filtered reads were assembled de novo using Unicycler, a hybrid assembly algorithm

Table 1. Antibiotic susceptibility of *Cutibacterium acnes* isolates from healthy individuals

Isolate	Clindamycin IZ (mm)	I	Tetracycline IZ (mm)	I	Azithromycin IZ (mm)	I	TMP-SMX IZ (mm)	I	Doxycycline IZ (mm)	I
<i>C. acnes</i> ATCC 6919	50.00 ± 0.00	S	50.00 ± 5.00	S	50.00 ± 10.00	S	39.50 ± 1.50	S	36.67 ± 2.89	S
Hn1-1	47.00 ± 3.00	S	43.33 ± 2.89	S	42.67 ± 2.52	S	40.00 ± 0.00	S	42.33 ± 2.52	S
Hn2	47.00 ± 0.00	S	54.00 ± 1.00	S	47.50 ± 7.50	S	40.00 ± 0.00	S	31.00 ± 1.00	S
Hn4	0.00 ± 0.00	R	44.33 ± 1.16	S	0.00 ± 0.00	R	33.00 ± 1.00	S	43.67 ± 1.53	S
Hn5-1	38.00 ± 5.20	S	15.00 ± 0.00	R	24.00 ± 1.00	S	39.50 ± 0.50	S	22.67 ± 2.52	S
Hn7-2	50.00 ± 0.00	S	40.00 ± 0.00	S	40.00 ± 10.00	S	31.00 ± 1.00	S	35.00 ± 5.00	S
Hn8-1	0.00 ± 0.00	R	49.00 ± 4.00	S	0.00 ± 0.00	R	38.50 ± 1.50	S	22.50 ± 2.50	S
Hn8-2	21.50 ± 1.50	S	52.00 ± 5.00	S	0.00 ± 0.00	R	29.67 ± 4.73	S	63.00 ± 3.00	S
Hn9-2	45.50 ± 0.50	S	27.00 ± 2.65	S	10.67 ± 2.52	R	45.00 ± 0.00	S	30.00 ± 0.00	S
Hn10-1	50.00 ± 3.61	S	32.00 ± 5.00	S	30.00 ± 5.00	S	35.00 ± 4.36	S	32.50 ± 2.50	S
Hn10-2	32.00 ± 0.00	S	51.00 ± 1.00	S	48.50 ± 1.50	S	30.00 ± 0.00	S	51.67 ± 2.89	S
Hn12-1	0.00 ± 0.00	R	46.33 ± 1.16	S	0.00 ± 0.00	R	42.00 ± 0.00	S	46.33 ± 1.16	S
Hn13	51.00 ± 1.00	S	48.67 ± 3.22	S	57.50 ± 2.50	S	42.00 ± 0.00	S	53.33 ± 5.77	S
Hn14	31.50 ± 0.50	S	55.67 ± 0.58	S	0.00 ± 0.00	R	30.00 ± 0.00	S	64.67 ± 3.22	S
Hn15-1	43.50 ± 0.50	S	38.00 ± 6.93	S	50.00 ± 0.00	S	40.00 ± 0.00	S	46.33 ± 3.22	S
Hn15-2	21.50 ± 1.50	S	50.00 ± 0.00	S	0.00 ± 0.00	R	41.00 ± 1.00	S	54.33 ± 0.58	S

Notes: IZ: inhibition zone diameter (mm); I: interpretation; S: susceptible; R: resistant. Susceptibility was interpreted according to EUCAST criteria for *Staphylococcus* spp.

specifically optimized for bacterial genome reconstruction.¹² The quality of the assembled genomes was comprehensively evaluated using QUAST based on key metrics, including total genome length, number of contigs, N50 value, and overall genome completeness.¹³

Functional annotation and molecular typing

The assembled genomes were functionally annotated using Prokka to identify structural genes, protein-coding sequences, RNA genes, and functional genomic regions.¹⁴ Genome structure visualization and comparative analysis were performed using the Proksee platform. Strain-level identification was subsequently conducted using multi-locus sequence typing (MLST), based on allele profiling of standardized housekeeping genes.

Antimicrobial resistance gene analysis

Genes and variants associated with antimicrobial resistance were identified using AMRFinderPlus,¹⁵ which detects resistance-associated genetic markers through comparison with a curated reference database.

This study was approved by the Ethics Committee of Biomedical Research, Can Tho University of Medicine and Pharmacy (Ref. No. 24.004/PCT-HIII). Written informed consent was obtained from the participants before enrolling in the study.

RESULTS AND DISCUSSION

Antibiotic susceptibility of *Cutibacterium acnes* isolates from healthy individuals

The results showed that most *C. acnes* isolates (n = 16, including 15 clinical isolates obtained from healthy individuals and one reference strain used as a control) were susceptible to antibiotics belonging to the macrolide, lincosamide and tetracycline classes; however, antibiotic resistance was still observed (Table 1). Notably, azithromycin exhibited the highest resistance rate, with 7 out of 16 isolates (43.75%) classified as resistant, while resistance to clindamycin (18.75%) and tetracycline (6.25%) was also detected, as indicated by the absence or marked reduction of inhibition zones (Figure 1). In contrast, all 16 isolates remained fully susceptible to doxycycline and TMP-SMX (100%), highlighting

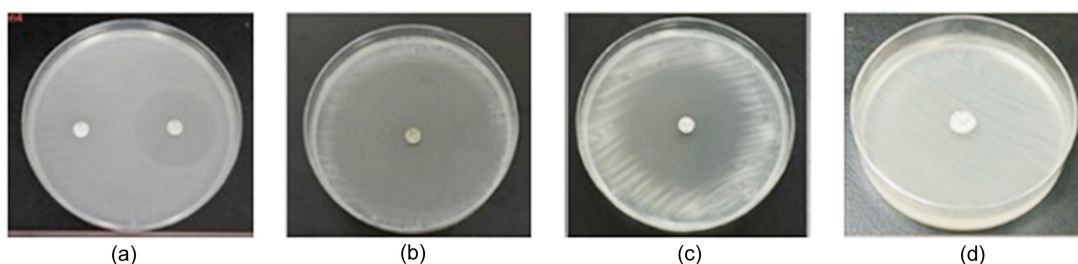


Figure 1. Inhibition zones of antibiotics against *Cutibacterium acnes* isolates obtained from healthy individuals
Note: (a) left - Clindamycin, right - TMP-SMX; (b) Doxycycline; (c) Tetracycline; (d) Azithromycin

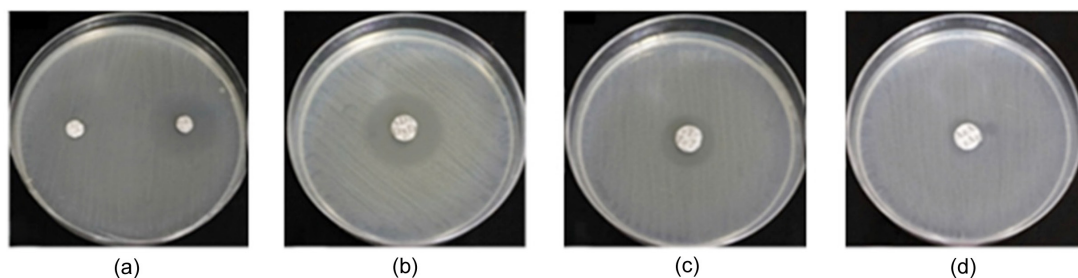


Figure 2. Inhibition zones of antibiotics against *Cutibacterium acnes* isolates obtained from mild acne patients
Note: (a) left - Clindamycin, right - TMP-SMX; (b) Doxycycline; (c) Tetracycline; (d) Azithromycin

Table 2. Antibiotic susceptibility of *Cutibacterium acnes* isolates from mild acne patients

Isolate	Clindamycin IZ (mm)	I	Tetracycline IZ (mm)	I	Azithromycin IZ (mm)	I	TMP-SMX IZ (mm)	I	Doxycycline IZ (mm)	I
Mn1	0.00 ± 0.00	R	44.00 ± 3.61	S	0.00 ± 0.00	R	38.00 ± 0.00	S	41.00 ± 9.00	S
Mn2-2	0.00 ± 0.00	R	65.00 ± 5.00	S	0.00 ± 0.00	R	39.00 ± 1.00	S	35.00 ± 0.00	S
Mn2-3	39.50 ± 5.50	S	47.33 ± 2.52	S	0.00 ± 0.00	R	32.50 ± 0.50	S	56.67 ± 5.77	S
Mn3-2	45.50 ± 0.50	S	52.50 ± 2.50	S	49.67 ± 2.52	S	35.00 ± 1.00	S	51.67 ± 2.89	S
Mn4	44.50 ± 0.50	S	24.00 ± 0.00	S	35.00 ± 0.00	S	27.50 ± 4.50	S	33.50 ± 0.50	S
Mn5-1	43.00 ± 1.00	S	67.50 ± 2.50	S	55.50 ± 0.50	S	44.00 ± 1.00	S	56.50 ± 0.50	S
Mn7-1	43.00 ± 0.00	S	20.00 ± 0.00	R	35.00 ± 0.00	S	35.00 ± 0.00	S	41.00 ± 1.00	S
Mn9-1	51.00 ± 1.00	S	30.00 ± 0.00	S	40.00 ± 0.00	S	38.00 ± 2.00	S	45.00 ± 0.00	S
Mn9-2	59.50 ± 0.50	S	60.00 ± 0.00	S	45.00 ± 0.00	S	47.50 ± 0.50	S	41.00 ± 3.61	S
Mn11-1	47.00 ± 0.00	S	45.00 ± 5.00	S	40.00 ± 5.00	S	38.00 ± 0.00	S	45.00 ± 0.00	S
Mn11-2	0.00 ± 0.00	R	12.33 ± 2.52	R	0.00 ± 0.00	R	27.00 ± 0.00	S	18.50 ± 1.50	R
Mn12-1	46.00 ± 0.00	S	45.00 ± 0.00	S	51.67 ± 2.89	S	35.00 ± 0.00	S	55.00 ± 0.00	S
Mn12-2	45.00 ± 5.00	S	55.00 ± 0.00	S	55.00 ± 0.00	S	39.00 ± 0.00	S	50.00 ± 0.00	S
Mn13-2	0.00 ± 0.00	R	60.00 ± 5.00	S	0.00 ± 0.00	R	39.00 ± 1.00	S	57.50 ± 2.50	S
Mn14-1	42.00 ± 2.00	S	16.00 ± 1.00	R	23.00 ± 0.00	S	34.00 ± 1.00	S	22.50 ± 2.50	S
Mn15-1	41.50 ± 0.50	S	48.67 ± 5.51	S	47.50 ± 2.50	S	37.00 ± 0.00	S	52.50 ± 2.50	S
Mn15-2	49.50 ± 0.50	S	20.67 ± 0.58	R	22.33 ± 2.52	S	39.50 ± 0.50	S	30.00 ± 0.00	S

Notes: IZ: inhibition zone diameter (mm); I: interpretation; S: susceptible; R: resistant. Susceptibility was interpreted according to EUCAST criteria for *Staphylococcus* spp.

substantial inter-strain variability in antibiotic resistance phenotypes among *C. acnes* isolates, consistent with previous reports describing heterogeneous resistance profiles across different phylogenetic lineages and clinical isolates.¹⁶

The detection of *C. acnes* strains resistant to azithromycin and clindamycin in a healthy population indicates that antibiotic resistance genes can persist and potentially disseminate even in the absence of overt disease.¹ This observation is concerning because it implies an accumulation of resistant bacteria in the community reservoir and underscores the need for vigilant monitoring of antibiotic use as well as systematic surveillance of the dynamics of resistant bacterial strains.³

Antibiotic susceptibility of *Cutibacterium acnes* isolates from mild acne patients

Seventeen *C. acnes* isolates were evaluated for susceptibility to five commonly used antibiotics, including clindamycin, tetracycline, azithromycin, TMP-SMX, and doxycycline (Table 2; Figure 2). All isolates were fully susceptible to TMP-SMX (100%), with inhibition zone diameters ranging from 27 ± 4.5 mm to 47.5 ± 2.5 mm. Doxycycline also showed high efficacy, with 94% of isolates (16/17) remaining susceptible. Susceptibility rates for clindamycin and tetracycline were both 82% (14/17 isolates), whereas azithromycin exhibited the lowest susceptibility rate at 76% (13/17 isolates). Overall, resistance to clindamycin, tetracycline, and azithromycin was observed in 22%-28% of the isolates.

The notable resistance rates observed for clindamycin, tetracycline, and particularly azithromycin indicate a declining effectiveness of several antibiotics commonly used in acne

treatment. These findings are consistent with previous studies reporting that prolonged use or monotherapy with clindamycin and azithromycin contributes to the selection and persistence of resistant *C. acnes* strains.¹ In contrast, the sustained susceptibility to TMP-SMX and doxycycline suggests that these agents remain effective therapeutic options. Collectively, these results underscore the importance of prudent antibiotic use and the implementation of antimicrobial resistance surveillance to limit the further spread of resistant *C. acnes* within the community.^{17,18}

Antibiotic susceptibility of *Cutibacterium acnes* isolates from moderate-severe acne patients

Twelve *C. acnes* isolates obtained from patients with moderate-severe acne were evaluated for susceptibility to five commonly used antibiotics, including clindamycin, tetracycline, azithromycin, TMP-SMX, and doxycycline (Table 3; Figure 3). Antibiotics belonging to the tetracycline class (tetracycline and doxycycline) and the trimethoprim-sulfamethoxazole combination (TMP-SMX) showed complete susceptibility, with all isolates (100%) classified as susceptible. The inhibition zone diameters ranged from 22.00 ± 0.00 mm to 62.67 ± 0.58 mm for tetracycline, from 27.67 ± 0.58 mm to 45.00 ± 0.00 mm for TMP-SMX, and from 29.33 ± 1.16 mm to 64.00 ± 0.00 mm for doxycycline. In contrast, four isolates (Sn3, Sn4-2, Sn14-1, and Sn14-2) exhibited resistance to both clindamycin and azithromycin, accounting for 33.33% of the tested isolates.

The present findings indicate that *C. acnes* isolates from patients with moderate-severe acne remain highly susceptible to TMP-

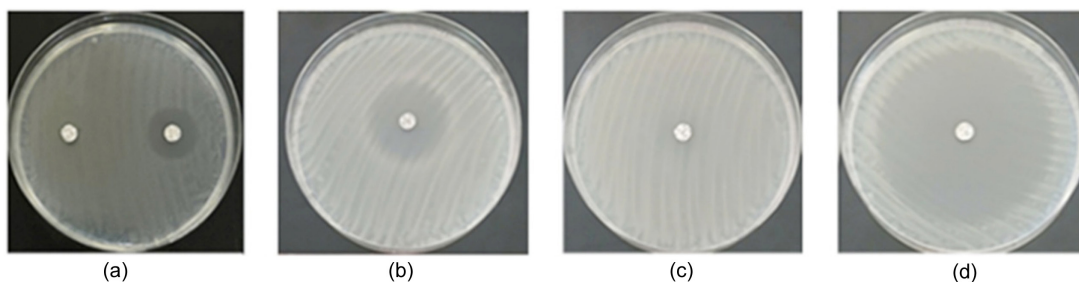


Figure 3. Inhibition zones of antibiotics against *Cutibacterium acnes* isolates obtained from moderate-severe acne patients

Note: (a) left - Clindamycin, right - TMP-SMX; (b) Doxycycline; (c) Tetracycline; (d) Azithromycin

Table 3. Antibiotic susceptibility of *Cutibacterium acnes* isolates from moderate-severe acne patients

Isolate	Clindamycin IZ (mm)	I	Tetracycline IZ (mm)	I	Azithromycin IZ (mm)	I	TMP-SMXIZ IZ (mm)	I	Doxycycline IZ (mm)	I
Sn1-1	48.67 ± 1.53	S	22.00 ± 0.00	S	37.67 ± 7.51	S	37.00 ± 0.00	S	43.67 ± 0.58	S
Sn3	0.00 ± 0.00	R	32.67 ± 2.52	S	0.00 ± 0.00	R	45.00 ± 0.00	S	32.33 ± 6.43	S
Sn4-2	0.00 ± 0.00	R	58.00 ± 0.00	S	0.00 ± 0.00	R	33.00 ± 9.85	S	49.00 ± 1.00	S
Sn9-1	41.00 ± 1.00	S	62.67 ± 0.58	S	65.00 ± 0.00	S	30.67 ± 0.58	S	64.00 ± 0.00	S
Sn9-2	40.00 ± 0.00	S	45.00 ± 3.00	S	45.00 ± 0.00	S	32.67 ± 2.52	S	51.67 ± 1.53	S
Sn10	50.00 ± 0.00	S	43.67 ± 1.53	S	45.00 ± 0.01	S	33.00 ± 0.00	S	48.00 ± 0.00	S
Sn11-1	39.00 ± 1.00	S	41.67 ± 0.58	S	33.67 ± 1.53	S	35.00 ± 0.00	S	37.67 ± 2.52	S
Sn11-2	42.00 ± 0.00	S	50.00 ± 0.00	S	46.67 ± 1.53	S	41.00 ± 0.00	S	48.00 ± 0.00	S
Sn12	43.67 ± 0.58	S	55.67 ± 0.58	S	51.00 ± 4.00	S	34.67 ± 0.58	S	49.00 ± 3.61	S
Sn13-2	49.00 ± 5.00	S	43.67 ± 1.53	S	50.33 ± 2.59	S	39.00 ± 6.00	S	48.33 ± 5.69	S
Sn14-1	0.00 ± 0.00	R	30.00 ± 0.00	S	16.00 ± 1.00	R	27.67 ± 0.58	S	29.33 ± 1.16	S
Sn14-2	8.33 ± 1.53	R	52.67 ± 0.58	S	0.00 ± 0.00	R	43.00 ± 0.00	S	52.67 ± 0.58	S

Notes: IZ: inhibition zone diameter (mm); I: interpretation; S: susceptible; R: resistant. Susceptibility was interpreted according to EUCAST criteria for *Staphylococcus* spp.

SMX, doxycycline, and tetracycline, suggesting that these antibiotics continue to be effective options for bacterial control. In contrast, the considerable resistance observed to clindamycin and azithromycin reflects a declining efficacy of antibiotics that have been widely and frequently used in acne treatment.^{1,7} The presence of resistant *C. acnes* strains in isolates derived from both diseased and healthy populations raises concerns regarding the accumulation and dissemination of antimicrobial resistance within the community and underscores the importance of prudent antibiotic use and strengthened resistance surveillance in clinical practice.^{1,5}

Optimization of sequencing data

All four samples exhibited high sequencing quality, and data cleaning using fastQ effectively improved read quality (Table 4). Prior to filtering, %Q30 values exceeded 91% for all samples, with three samples (Hn4, Hn13, and Mn1) showing values above 93%, indicating reliable raw data. After adapter trimming and removal of low-quality reads, %Q30 increased across all samples, confirming the efficiency of the cleaning process. Approximately 3% of reads were removed, suggesting low background noise and preservation of most high-quality reads for downstream analyses.

GC content (%GC) remained highly stable before and after filtering, with minimal variation (0.05%-0.08%), indicating the absence of nucleotide composition bias. The similar %GC values across samples (59.5%-60.2%) are consistent with the genomic characteristics typically reported for *C. acnes* and may reflect overall genomic similarity among the analyzed isolates.

Genome assembly

Key statistics describing the de novo assembly quality of four *C. acnes* genomes (Hn4, Hn13, Mn1, and Sn3) are summarized in Table 5. In terms of fragmentation, Mn1 (13 contigs) and Hn13 (14 contigs) exhibited the lowest contig numbers, indicating the most complete assemblies; notably, all contigs in Hn13 were longer than 1,000 bp. Assemblies of Hn4 (24 contigs) and Sn3 (19 contigs) showed slightly higher fragmentation but remained acceptable.

Table 4. Sequencing quality metrics before and after data cleaning

Sample	No. of reads	Total bases (bp)	%GC	%Q30
Before quality filtering				
Hn4	2,818,280	422,742,000	60.09	94.47
Hn13	3,674,966	551,244,900	60.06	93.71
Mn1	8,140,776	1,221,116,000	60.17	95.41
Sn3	3,578,368	536,755,200	59.54	91.75
After quality filtering				
Hn4	2,743,442	410,671,862	60.17	95.23
Hn13	3,578,334	535,627,374	60.13	94.48
Mn1	7,924,356	1,184,617,000	60.23	96.18
Sn3	3,474,936	519,854,798	59.6	92.59

Note: %Q30 represents the percentage of bases with a Phred quality score ≥ 30

Table 5. De novo genome assembly statistics

Metric	Hn4	Hn13	Mn1	Sn3
Number of contigs	24	14	13	19
Number of contigs >1,000 bp	17	14	12	17
%GC	60.04	60.06	60.17	60.1
Total contig length (bp)*	2,541,425	2,470,568	2,483,967	2,516,009
Longest contig length (bp)	572,461	683,404	873,047	722,801
N50 (bp)	323,417	288,109	717,330	216,077
L50	3	3	2	3

Note: *Calculated based on contigs $\geq 1,000$ bp

The estimated genome sizes were highly consistent across isolates, ranging from approximately 2.47 Mb to 2.54 Mb, with stable GC contents (60.04%-60.17%), consistent with raw sequencing data and indicating the absence of assembly-induced nucleotide bias. Regarding continuity, Mn1 showed superior performance with an N50 of 717,330 bp and an L50 of 2 and also contained the longest contig (873,047 bp). Hn4 and Hn13 demonstrated good continuity, whereas Sn3 had the lowest N50 (216,077 bp). Overall, all four genomes were assembled with high quality and were suitable for downstream functional annotation, with Mn1 representing the highest-quality assembly.

Multilocus sequence typing based on whole-genome data

MLST analysis using the *pacnes_3* scheme (eight loci: *aroE*, *atpD*, *gmk*, *guaA*, *lepA*, *sodA*, *tly*, and *CAMP2*) was conducted on four *C. acnes*

genomes (Hn4, Hn13, Mn1, and Sn3). All isolates shared a common genetic background but differed in sequence types (STs). Hn4 was assigned to ST53 and Sn3 to ST1, whereas Hn13 and Mn1 could not be assigned to existing STs, suggesting potential novel STs or variants closely related to known lineages (Table 6).

All isolates shared *aroE*(1) and *lepA*(1) alleles, indicating strong conservation of housekeeping genes, while variability was mainly observed at *gmk*, *guaA*, *sodA*, *tly*, and *CAMP2*. Sn3 (ST1) displayed a typical phylotype IA1 allele profile, which represents a lineage commonly associated with acne lesions, heightened inflammatory responses, and increased virulence potential,^{19,20} and is frequently reported in severe acne with increased virulence or antibiotic resistance potential.⁹ In contrast, ST53 (Hn4) is commonly associated with phylotypes IB/IA2 and has been linked to healthy skin or opportunistic infections.^{21,22}

Table 6. MLST-based sequence typing results of four *Cutibacterium acnes* genomes

Isolate	MLST scheme	Sequence type (ST)	Allele profile
Hn4	pacnes_3	53	<i>aroE</i> (1), <i>atpD</i> (1), <i>gmk</i> (9), <i>guaA</i> (4), <i>lepA</i> (1), <i>sodA</i> (4), <i>tly</i> (8), <i>CAMP2</i> (6)
Hn13	pacnes_3	—	<i>aroE</i> (1), <i>atpD</i> (~1), <i>gmk</i> (1), <i>guaA</i> (3), <i>lepA</i> (1), <i>sodA</i> (1), <i>tly</i> (1), <i>CAMP2</i> (1)
Mn1	pacnes_3	—	<i>aroE</i> (1), <i>atpD</i> (1), <i>gmk</i> (1), <i>guaA</i> (5), <i>lepA</i> (1), <i>sodA</i> (~4), <i>tly</i> (8), <i>CAMP2</i> (2)
Sn3	pacnes_3	1	<i>aroE</i> (1), <i>atpD</i> (1), <i>gmk</i> (1), <i>guaA</i> (3), <i>lepA</i> (1), <i>sodA</i> (1), <i>tly</i> (1), <i>CAMP2</i> (1)

Notes: The MLST analysis was performed using the pacnes_3 scheme comprising eight loci (*aroE*, *atpD*, *gmk*, *guaA*, *lepA*, *sodA*, *tly*, and *CAMP2*); “—” indicates that the sequence type could not be assigned based on the current MLST database; Approximate allele matches are indicated by “~”

Table 7. Overview of genomic components of four *Cutibacterium acnes* isolates

Sequence type	Hn4	Hn13	Mn1	Sn3
CDS (Coding sequences)	2,354	2,289	2,304	2,343
tRNA	47	46	46	46
rRNA	3	3	4	6

The inability to assign STs to Hn13 and Mn1 is consistent with the high discriminatory power of MLST and the frequent emergence of new allelic profiles during lineage diversification.²³ Mn1 exhibited a mosaic allele pattern combining features of ST1 and ST53, suggesting possible allelic diversification within related *C. acnes* lineages rather than assignment to a single established ST.²⁴ Moreover, diversity at the *tly* and *CAMP2* loci, genes implicated in virulence, immune modulation, and biofilm formation, suggests potential phenotypic differences among *C. acnes* lineages.^{25,26} The coexistence of ST1, ST53, and putative novel STs reflects a complex population structure that may influence inflammatory outcomes on human skin.²⁰

Basic functional annotation

Structural genome annotation of four *C. acnes* isolates (Hn4, Hn13, Mn1, and Sn3) revealed a high level of conservation in core genomic features (Table 7). The number of predicted coding sequences ranged from 2,289 to 2,354, while tRNA gene counts were highly conserved (46-47 genes). In contrast, rRNA gene copy numbers varied more substantially, ranging from 3-6 among the isolates. Overall, the predicted proteome size

and translational machinery were largely stable across the isolates, consistent with the conserved genomic organization commonly reported for *C. acnes*, whereas minor differences in CDS and rRNA counts suggest strain-level variation.

Circular genome maps generated using Proksee demonstrated highly similar genome organization across the four isolates, characterized by dense CDS distribution and interspersed tRNA/rRNA regions, consistent with the annotation statistics (Figure 4).

Annotation of virulence and antimicrobial resistance genes

Genome-wide annotation of four *C. acnes* isolates was performed using the ABRicate pipeline on the Galaxy platform against the CARD, NCBI AMR, and VFDB databases. Using minimum thresholds of 80% nucleotide identity and 80% reference sequence coverage, no antimicrobial resistance or virulence-associated genes were detected in the analyzed genomes. In contrast, analysis with AMRFinderPlus identified a single antimicrobial resistance gene, *erm*(51), exclusively in the Mn1 genome (Table 8). The *erm*(51) gene was located on a chromosomal contig and showed complete sequence coverage with high similarity to the reference sequence, suggesting the presence of an intact resistance-associated gene. No additional resistance genes or resistance-associated mutations were identified in the remaining genomes. The presence of *erm*(51) in Mn1 was consistent with the antimicrobial susceptibility profile observed for this isolate.

The detection of *erm*(51) as the sole antimicrobial resistance gene in the genome of

C. acnes Mn1, in the absence of other resistance markers such as *tet(W)/tet(M)* (tetracycline resistance), *erm(X)* (macrolide–clindamycin resistance), *gyrA/parC* mutations (fluoroquinolone resistance), or *rpoB* mutations (rifampicin

resistance), suggests a relatively narrow resistance spectrum primarily restricted to the macrolide–lincosamide–streptogramin B (MLSB) group, rather than a broad multidrug-resistant (MDR) phenotype, typically defined as resistance to

Table 8. Annotation results of antimicrobial resistance genes identified in the Mn1 genome

Parameter	Description
Contig ID	12
Start position	763
End position	1551
DNA strand	+
Target gene	<i>erm(51)</i>
Gene product	23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(51)
Antibiotic class	Lincosamide/macrolide/streptogramin
Antibiotics affected	Azithromycin, clarithromycin, clindamycin, erythromycin
Gene length (bp)	263
Reference coverage (%)	100
Reference identity (%)	99.62
Alignment length (bp)	263

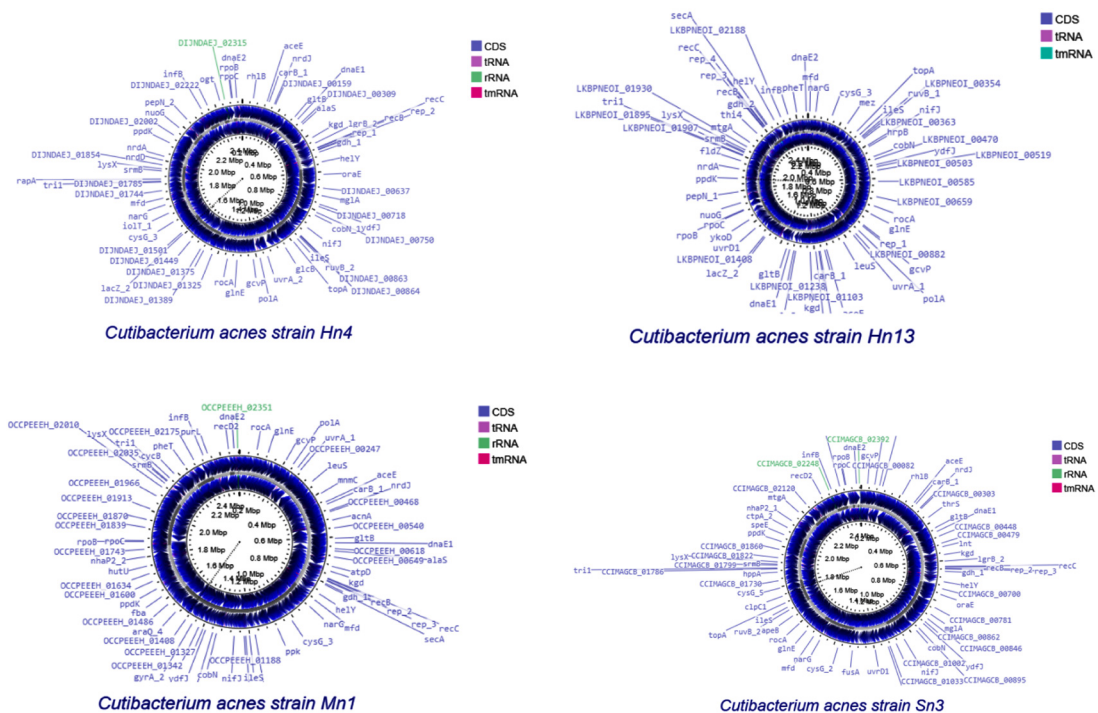


Figure 4. Circular genome maps of four *Cutibacterium acnes* isolates

Notes: Circular genome maps of *Cutibacterium acnes* strains Hn4, Hn13, Mn1, and Sn3. Colored rings represent coding sequences (CDS), *tRNA*, *rRNA*, and *tmRNA* genes. Gene annotations displayed on the outermost ring indicate the distribution of functional coding regions across the genomes of each strain

multiple classes of antimicrobial agents, as reported for some *C. acnes* or *Cutibacterium* spp. strains.⁸

Over the past decade, increasing resistance to macrolides and clindamycin in *C. acnes* has been widely reported, with marked geographic variability. Epidemiological data from Jordan showed resistance rates of 73% to erythromycin and 59% to clindamycin,⁶ while recent global analyses indicate that more than 50% of *C. acnes* isolates are resistant to these antibiotics, exceeding 90% in certain regions.^{5,6} These trends are consistent with recent reviews emphasizing prolonged macrolide use as a major driver of resistance selection.²⁷

Mechanistically, MLSB resistance in *C. acnes* is mainly mediated by point mutations in 23S rRNA or acquisition of *erm* methylase genes via plasmids or other mobile genetic elements.⁹ Aoki et al. described the transferable plasmid pTZC1 carrying *erm*(50) and *tet*(W), highlighting the role of plasmid-borne *erm* genes in the emergence of MDR strains. In this context, *erm*(51), a member of the Erm 23S rRNA methyltransferase family, has been characterized as a functional MLSB resistance determinant with potential for horizontal transfer.²⁷

Notably, the Mn1 genome lacked resistance markers for tetracyclines or rifampicin that have been reported in MDR *C. acnes* strains,^{9,28} suggesting that macrolide–lincosamide resistance may currently be evolving independently without progression to a broad MDR phenotype. Nevertheless, the presence of an *erm* gene remains epidemiologically relevant, as it may facilitate further resistance accumulation under sustained antibiotic pressure.⁸ Clinically, these findings reinforce the importance of antimicrobial stewardship by limiting unnecessary macrolide and clindamycin exposure and prioritizing tetracycline-class antibiotics or non-antibiotic therapeutic approaches in accordance with current acne management guidelines.¹

CONCLUSION

This study provides an integrated assessment of antibiotic susceptibility patterns and genomic characteristics of *C. acnes* isolates obtained from healthy individuals and patients

with different severities of acne vulgaris. Phenotypic analysis showed that most isolates remained highly susceptible to tetracycline-class antibiotics and trimethoprim, whereas resistance to macrolides and clindamycin was detected, particularly among moderate–severe acne isolates. Whole-genome sequencing combined with MLST revealed notable genetic diversity, including both established sequence types and putative novel variants. The identification of a single resistance-associated gene, *erm*(51), consistent with the observed resistance phenotype, suggests that the analyzed *C. acnes* population has not yet developed widespread multidrug resistance. However, interpretation of these findings should consider the relatively small number of sequenced isolates included in this study. Overall, these results highlight the value of integrating phenotypic susceptibility testing with genomic analysis to support ongoing resistance surveillance programs and evidence-based antibiotic stewardship in acne management.

ACKNOWLEDGMENTS

The authors sincerely thank the Institute of Food and Biotechnology, Can Tho University, Vietnam, for their kind support and for providing access to their laboratories. The authors also thank Can Tho Hospital of Dermato-Venereology for allowing sample collection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

XMT and DNT conceptualized the study. DNT developed the methodology. XMT performed the software-related work. XMT and LTTN conducted the formal analysis. LTTN and DNT validated the data. LDHB and DNT carried out the investigation. XMT, PXH, and TNN performed data curation. PXH, LTTN, LDHB, and TNN contributed to data visualization. LTTN, XMT, LDHB, and TNN interpreted the data. PXH and DNT supervised the study. XMT prepared the original draft. XMT, PXH, and DNT wrote, reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The genome assemblies and associated metadata generated in this study have been submitted to the NCBI database under BioProject accession number PRJNA1466956. The corresponding BioSample accession numbers are SAMN60158190 (Hn4), SAMN60158191 (Hn13), SAMN60158192 (Mn1), and SAMN60158193 (Sn3). The additional datasets generated and/or analysed during the current study are included in the article and the rest can be obtained from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Biomedical Research, Can Tho University of Medicine and Pharmacy (Ref. No. 24.004/PCT-HIII).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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