ORIGINAL RESEARCH

First Identification of a Patient with Prosthesis-Related Infection Caused by an MCR-1.1-Producing ST131 *Escherichia coli* After Rhinoplasty

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Background: The most common procedure of rhinoplasty is the implantation of a synthetic prosthesis. However, the complications, especially postoperative infection, could lead the suboptimal aesthetic outcome, economic losses and health threats. There is currently little literature providing an incidence of rhinoplasty infection and microbiological and antimicrobial resistance situations.

Methods: Therefore, we performed a retrospective observational study which included 173 patients who received a rhinoplasty from 1 January 2015, to 31 December 2019, in the department of plastic surgery of a tertiary hospital in Guangzhou, China. The samples from the infection site were collected and performed the bacterial culture. The antimicrobial susceptibility testing was performed by VITEK and minimum inhibition concentration testing. The whole-genome sequencing was performed by Illumina Hiseq4000 platform.

Results: We found that eight (4.6%) patients were infected by *S. aureus* (6), *E. raffinosus* (1) and *E. coli* (1), of which are susceptible to most antimicrobials. Remarkably, *E. coli* RS1231 was resistant to colistin and polymyxin B which conferred by *mcr-1.1* locating on an IncI2 plasmid with 59,170-bp sequence length. Through sequence comparison, we speculate that the pRS1231S-MCR-1 was derived from animal sources. Besides, *E. coli* RS1231 belongs to ST131 O25:H4-fimH22 pandemic subclone and phylogroup B2, which can induce a broad variety of infections.

Conclusion: Our study provided a rhinoplasty infection incidence, microbiological and antimicrobial resistance prevalence data, and revealed, to our knowledge, the first case of postoperative infection of rhinoplasty by *mcr-1.1*-positive, highly susceptible, and remarkably virulent *E. coli* isolate.

Keywords: rhinoplasty, colistin resistance, ST131, *mcr-1.1*, *Escherichia coli*, horizontal gene transfer, virulence

Introduction

The most common procedure of rhinoplasty is the implantation of synthetic prosthesis, which is popular in East Asia. However, as a surgical procedure, postoperative complications are inevitable. The common postoperative complications include displacement, extrusion, inflammation, iatrogenic injury, scarring and infection.[1] Although the infection after rhinoplasty is uncommonly reported, some processes, like osteotomies, performed during rhinoplasty breach the periosteum or

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nasal mucosa, creating a potential route for the spread of infection,[2,3] which can lead to the failure of plastic surgery, even increase the risk of systemic infections. The basic treatment options include continued observation, antimicrobial therapy and removal of the implant; however, some therapies could influence the aesthetics of the patients, which increases the financial cost and bring the psychological blow.[1,4]

Staphylococcus aureus and various *streptococci* are the main colonization bacteria and encountered pathogens associated with postoperative infections in plastic surgery.[3] To prevent postoperative infection, the use of cefazolin as a single dose is the most recommended antimicrobial for preoperative prophylaxis.[4] However, the emergence of infrequent pathogens in rhinoplasty infections, especially multidrug resistance (MDR) *Enterobacteriaceae*, is threatening the effect of preoperative prophylaxis and the effect of antimicrobial chemotherapy.[3]

Based on the routine surveillance, we retrospectively observed the patients with nasal prosthesis-related infections from 1 January 2015 to 31 December 2019 in a tertiary hospital in Guangzhou, China. The results showed that the *Staphylococcus aureus* was the most common bacteria of the patients with postoperative infection after rhinoplasty. Specially, we found a patient, who received implantation of the synthetic prosthesis, infected with a colistin-resistant *Escherichia coli* isolate. Besides, we illustrated the genomic characteristics of this *Escherichia coli* isolate through next-generation sequencing (NGS) and confirmed mobile colistin resistance gene *mcr-1.1*, which was frequently reported in China with a high prevalence in food-producing animals.[5]

Materials and Methods
Sample Collection
For the purpose of routine monitoring of the patients with prosthesis-related infection after rhinoplasty, this study included the patients, who received rhinoplasty during a 5-year period (1 January 2015 to 31 December 2019) from the department of plastic surgery of a tertiary hospital in Guangzhou, China. The patients with rhinoplasty infection were selected for microbiological analysis. The pus samples were collected from infection site and prosthesis of these patients using nose swab and sent to the department of clinical laboratory for bacterial culture, species identification and antimicrobial susceptibility testing. Oral

consent for the inclusion of research analysis for the patients was obtained.

Bacteria Isolation and Species Identification
The samples were plated on blood agar and incubated overnight at 37°C. Three of the culturable isolates were randomly selected and then subjected to species identification using VITEK 2.0 system (bioMérieux, Durham NC, USA) based on the analytical profile index. One of the isolates was subsequently subjected to the species identification verifying by 16S rDNA sequencing with PCR primers (27F: 5′ AGAGTTTGATCCTGGCTCAG 3′; 1429R: 5′ GGTTACCTTGTTACGACTT 3′). The species of *E. coli* RS1231S isolate was reconfirmed by MALDI-TOF (API 20E, bioMérieux, France).

Antimicrobial Susceptibility Testing (AST)
The obtained isolates were subjected to antimicrobial susceptibility testing using VITEK 2.0 system (bioMérieux, Durham NC, USA) using AST N-335 for gram-negative isolates (amikacin, cefepime, cefoperazone/sulbactam, ceftazidime, colistin, doxycycline, minocycline, meropenem, ciprofloxacin, levofloxacin, imipenem, tigecycline, piperacillin/tazobactam, ticarcillin clavulanic acid, aztreonam, tobramycin, trimethoprim/sulfamethoxazole) and AST GP67 (ampicillin, oxacillin, ciprofloxacin, clindamycin, gentamycin, levofloxacin, moxifloxacin, macrodantin, quinupristin/dalfopristin syncercid, rifampicin, tetracycline, trimethoprim/sulfamethoxazole, vancomycin, erythromycin, linezolid, tigecycline) for gram-positive isolates. To confirm the AST of RS1231S isolates, we performed minimum inhibitory concentrations (MICs) for colistin, polymyxin B, tigecycline, ampicillin, amoxicillin-clavulanate, cefotaxime, ceftazidime, cefepime, gentamicin, amikacin, ertapenem, imipenem, meropenem, fosfomycin, nitrofurantoin, and ciprofloxacin with three replications, using the agar dilution method excepted for colistin, polymyxin B and tigecycline which instead used the broth dilution method in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2019). *E. coli* ATCC 25,922 was used as the standard reference strain. We interpreted the results in accordance with the CLSI breakpoints (CLSI 2019).

Next-Generation Sequencing
DNA of *E. coli* RS1231S was extracted using PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA)

following the manufacturer's protocol. Genome library was prepared with 150-bp paired-end-read format and sequenced using Illumina HiSeq 4000 instrument (Illumina, Inc., San Diego, CA, United States) by Biomarker Technologies company. Sequencing data of raw sequence reads have been deposited with the NCBI (BioProject accession: PRJNA608869).

Bioinformatics Analysis

Raw reads were qualified using fastqc, trimmed and filtered with Trimmomatic to remove primer, adaptor and low-quality reads.[6,7] De novo genome assembly was performed using SPAdes Genome Assembler v3.9.0.[8] Assembled draft genome sequences were assessed by QUAST and annotated with RAST.[9,10] The antimicrobial resistance genes (ARGs) and virulence factors (VFs) were analyzed using Blastn[11] with e-value<1e-5 and identity>90%, based on comprehensive antimicrobial resistance database 3.0.7 (CARD) and virulence factor database (VFDB).[12,13] The acquired ARGs and plasmid replicon type was identified by ResFinder and PlasmidFinder v2.2 was imbedded in the Center for Genomic Epidemiology (CGE; https://cge.cbs.dtu.dk/services/) with default setting.[14,15] Multilocus sequence typing (MLST) of the E. coli was identified using In Silico typing tool based on the 7 housekeeping genes (adk, fumC, gyrB, icd, mdh, purA, recA) in Enterobase v.1.1.2.[16] The mcr-1.1-harboring plasmid was closed by polymerase chain reaction (PCR) and sanger sequencing (primers: F-'GACAGTACAGGGGCGATAC', R-'CGGCTGATTG TCATTTTGCG'; Annealing temperature: 59°C; Extension time: 50s; Sequence length: 370-bp). The phylogenetic tree was constructed by CSIPhylogeny using Escherichia coli str. K-12 substr. MG1655 (NC_000913.2) as the reference genome with default parameters, and visualized by iTOL.[17]

Results

Bacterial Prevalence of Prosthesis-Related Infection After Rhinoplasty

In this retrospective study, we included 173 patients who received rhinoplasty from 1 January 2015 to 31 December 2019. The average age of these patients was 31.2 (range: 20–51 years) and most of them are female (95.4%, 165/173). Among them, 8 (4.6%) patients had prosthesis-related infection after rhinoplasty. All these patients had local symptoms of swelling, extrusion,

inflammation and pain without symptoms of systemic infection. The results of bacterial culture and species identification revealed that these eight patients were infected by several bacterial species, including S. aureus (75.0%, 6/8), E. raffinosus (12.5%, 1/8), E. coli (12.5%, 1/8) (Tables 1 and 2).

Antimicrobial Resistance Spectrum

We performed AST for Gram-negative and Gram-positive bacteria, respectively. More than half of S. aureus and E. raffinosus isolates were resistant to ampicillin (85.7%, 6/7), erythromycin (57.1%, 4/7) and clindamycin (42.9%, 3/7), while all were susceptible to the most antimicrobials, including tigecycline, vancomycin, linezolid. The rest of the tested antimicrobials were kept low in resistance rate (Table 1). Specially, we found that the E. coli RS1231S isolate was only resistant to colistin (MIC = 8 μg/L) (Table 2).

Epidemiological Characteristics of the Patient Infected by Colistin-Resistant E. coli RS1231S

The medical record showed that the patient infected by colistin-resistant E. coli RS1231S isolate is a 48 years old female who underwent primary nasal prosthesis implantation on 7 June 2016. Before the surgery, the patient has received cefazolin as a preoperative prophylaxis antimicrobial. The patient was admitted to the First Affiliated Hospital of Guangzhou Medical University on 28 July 2016, complaining of 5 days fever (up to 37.9° C) with redness, swelling and pain of the nose. Clinical laboratory findings on admission showed that white blood cell count was 12.31×10^9/mL (79.8% neutrophils granulocyte) and C-reactive protein was 31.22 mg/L, which indicated that the patient could be infected by bacterial. Then, the pus samples of the synthetic prosthesis were sampled and sent to the clinical laboratory for bacterial culture. The empirical therapy of intravenous ceftriaxone (1.0g qd) was administered for the patient, and the symptom was relieved after 5 days of treatment.

Genomic Characteristics of E. coli RS1231S Isolate

To explore the colistin resistance mechanism of E. coli RS1231S isolate, we performed NGS to investigate the genomic feature of this isolate. We obtained 20,944,110 paired-end 150-bp reads which produced a 4.98Mb

Table 1 Clinical Information, Microbiological and Antimicrobial Resistance Spectrum of Patients with Gram-Positive Bacterial Infection

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (years)	46	34	34	32	40	39	41
Gender	Female	Female	Female	Female	Female	Female	Female
Bacterial species	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*	*E. raffinosus*
Isolate number	LO143	LP21	FI213	FI223	FI549	FI712	RI15
Admission time	3 March 2015	12 August 2015	18 May 2017	11 May 2017	28 May 2018	1 Nov 2018	17 June 2019
Antimicrobial resistance profile							
Ampicillin	R	R	S	R	R	R	R
Oxacillin	S	R	S	S	S	S	R
Clindamycin	R	R	S	S	R	S	S
Gentamycin	S	S	S	S	S	S	R
Ciprofloxacin	S	S	S	S	S	S	S
Levofloxacin	S	S	S	S	S	S	S
Moxifloxacin	S	S	S	S	S	S	S
Macrodantin	S	S	S	S	S	S	S
Quinupristin/dalfopristin syncercid	S	S	S	S	S	S	S
Rifampicin	S	S	S	S	S	S	S
Tetracycline	S	R	S	S	S	S	R
Tigecycline	S	S	S	S	S	S	S
Trimethoprim/sulfamethoxazole	S	R	S	S	S	S	R
Vancomycin	S	S	S	S	S	S	S
Erythromycin	R	R	S	R	S	S	R
Linezolid	S	S	S	S	S	S	S

Abbreviations: S, susceptible; R, resistance.

genome with 66 contigs and 50.6% GC content. *In silico* typing revealed that *E. coli* RS1231S belongs to ST131 O25:H4-fimH22 pandemic subclone and phylogenetic group B2, which is commonly regarded as an infection pathogen.[18,19] According to the ResFinder annotation, *E. coli* RS1231S harbored three acquired ARGs of mobile colistin resistance gene *mcr-1.1*, tetracycline resistance gene *tetA* and macrolides resistance gene *mdfA* (Table 2). Through VFDB screen, we identified 63 *E. coli* associated virulence genes, which involved in the categories of adherence (25.0%, 20/80), autotransporter (53.75%, 43/80), secretory system (18.75%, 15/80) and toxin (2.5%, 2/80) (Table 2). To investigate the potential sources of *E. coli* RS1231S isolate, we searched several genomes of MCR-1.1-producing *E. coli* isolate among animal, human and the environment from the publicly available database (Supplement Table 1). In the phylogenetic tree, *E. coli* RS1231S isolate and the most of infection-related *E. coli* genomes were highly correlated within a single node (Figure 1), which indicated that these infection-related

isolates were genetically different from isolates from other sources.
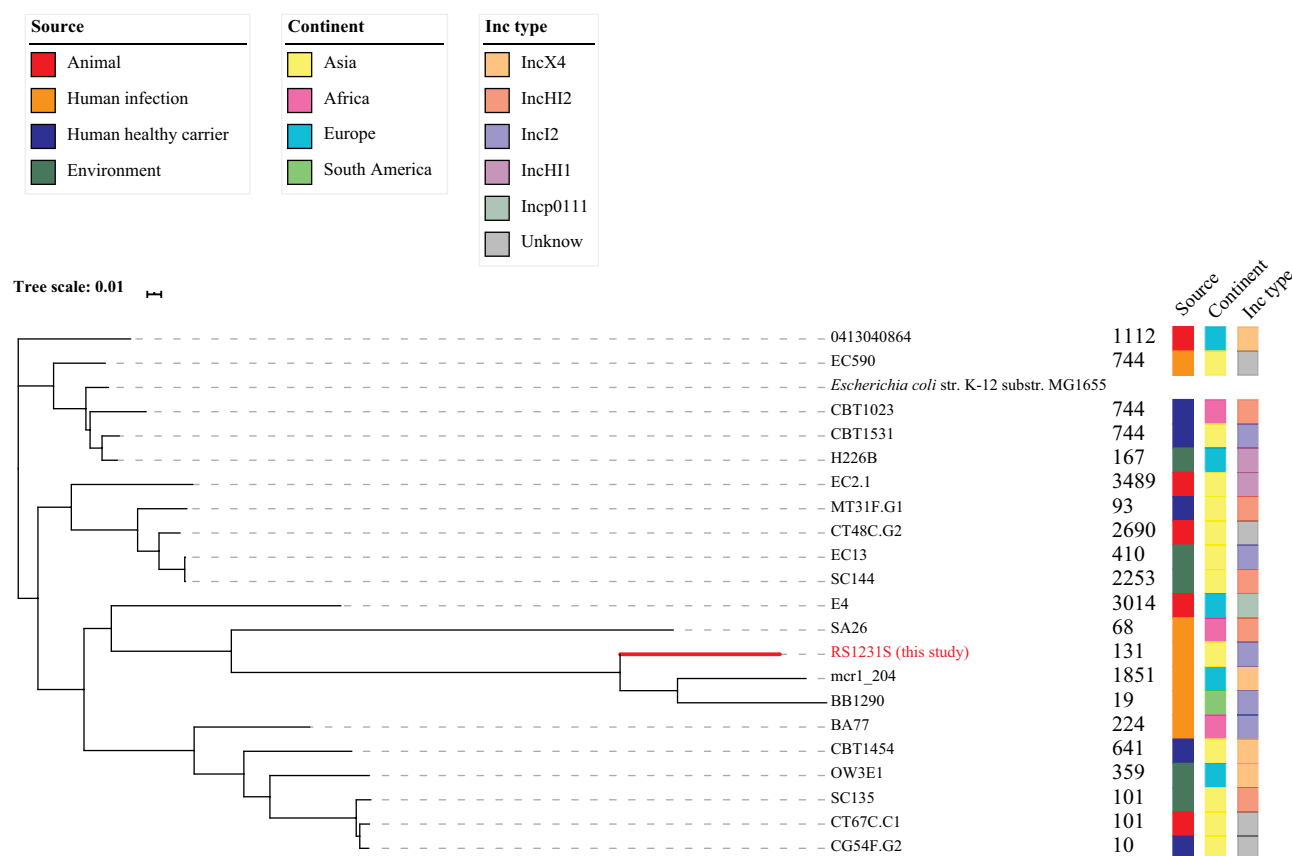
Plasmidome of *E. coli* RS1231S Isolate

The plasmid replicon typing results showed that *E. coli* RS1231S contains six Inc types (IncFIB, IncFIC, IncI2, ColpVC, ColRNAI, ColMG828), which consistent with previous knowledge that ST131 plasmidome consisted mostly of IncF and Col-like plasmids,[18,20] excepted IncI2 plasmid, indicating *E. coli* RS1231S belongs a pandemic subclone, which harbored various virulence factors with pathogenicity. Notably, we found that *mcr-1.1* gene was located on the same contig with IncI2 replicon. Therefore, PCR and Sanger sequencing were performed to close the IncI2-*mcr-1.1*-harboring contig, which produced a 59,170-bp sequence as pRS1231S-MCR-1 (Figure 2). The pRS1231S-MCR-1 contained 84 coding DNA sequences (CDSs) and only one antimicrobial resistance gene *mcr-1.1* was confirmed. The comparison of the first report *mcr-1.1*-harboring IncI2 plasmid pHNSHP45 (Accession

Table 2 Clinical Information, Microbiological, Antimicrobial Resistance and Genomic Characteristics of the Patient Infected by Colistin-Resistant *E. coli* RS1231S Isolate

	Patient 8		
Age (years)	48		
Gender	Female		
Bacterial species	*E. coli*		
Isolate number	RS1231S		
Admission time	28 July 2017		
MLST	ST131 (adk-53, fumC-40, gyrB-47, icd-13, mdh-36, purA-28, recA-29)		
Inc replicons	IncFIB, IncFIC, IncI2, ColpVC, ColRNAI, ColMG828		
Phylogroup	B2		
Serotype	O25:H4-fimH22		
Acquired ARGs (ResFinder)	*mdfA, mcr-1.1, tetA*		
Virulence factors (VFDB)	*ecpA, ecpB, ecpC, ecpD, ecpE, eaeH, hcpA, hcpB, hcpC, papC, papD, papH, fimA, fimC, fimD, fimE, fimF, fimG, fimH, fimI, cah, ehaB, espC, tsh, upaG/ehaG, vat, ibeA, ibeB, ibeC, CLASS, iucA, iucB, iucC, iucD, iutA, chuA, chuS, chuT, chuU, chuW, chuX, chuY, sitA, sitB, sitC, sitD, iroB, iroC, iroD, iroE, iroN, fyuA, irp1, irp2, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, usp, hlyE/clyA*		
Antimicrobial resistance spectrum			
Piperacillin/tazobactam	S	Ampicillin	R
Ticarcillin clavulanic acid	S	Amoxicillin–clavulanate	S
Cefepime	S	Cefotaxime	S
Ceftazidime	S	Gentamicin	S
Cefoperazone/sulbactam	S	Ertapenem	S
Imipenem	S	Fosfomycin	S
Meropenem	S	Nitrofurantoin	S
Colistin	R	Tobramycin	S
Polymyxin B	R	Minocycline	S
Amikacin	S	Tigecycline	S
Ciprofloxacin	S	Aztreonam	S
Levofloxacin	S	Trimethoprim/sulfamethoxazole	S
Doxycycline	S		

Abbreviations: S, susceptible; R, resistance.

Number KP347127.1) and pRS1231S-MCR-1 demonstrated a highly similar identity (99.99%) and coverage (92%), whereas pRS1231S-MCR-1 lost an insertion sequence IS*683*, an IS*Apl1* that located on the upstream of *mcr-1.1*, and a *pilV* associated with pilin assembly (Figure 1). Additionally, we found some loss of segments of Transposon Tn*6330* (IS*Apl1-mcr-1.1-pap2*-IS*Apl1*) was associated with *mcr-1.1* mobilization.[21] However, there is no IS*Apl1* was found around *mcr-1.1* on pRS1231S-MCR-1.

Discussion

The postoperative infection after rhinoplasty was uncommon and uncommonly reported, but can influence the suboptimal aesthetic outcome, economic losses and health threats.[1] With the development of antibiotic resistance, it is important to monitor the microbiological and antimicrobial resistance for postoperative infection of rhinoplasty.[3,22]

In our study, we investigated the microbiological and antimicrobial resistance prevalence of 8 out of 173 patients who undergone rhinoplasty and had a postoperative infection, which is consistent with a low incidence in other countries (0–5%). Indeed, *S. aureus* is the most common pathogen found in rhinoplasty infections,[3] whereas the *E. raffinosus* and *E. coli* were rarely reported. The antimicrobial resistance testing showed that most of *S. aureus* isolates and *E. raffinosus* isolate were resistant to ampicillin, erythromycin and clindamycin but generally susceptible to other antimicrobials, revealing the AMR situation of Gram-positive pathogen infection of rhinoplasty is not severe relatively. However, an *E. coli* RS1231S isolate was found in an infected patient and showed resistance

Figure 1 Phylogeny of MCR-1.1-producing isolates from various sources, including *E. coli* RS1231S. The tree was constructed by CSIPhylogeny based on draft genome for each isolate based on *Escherichia coli* str. K-12 substr. MG1655. The tree was visualized using iTOL. The source, continent and plasmid Inc type for each isolate were shown on the right of the tree by colored squares. The *E. coli* RS1231S isolate found in this study was shown as red text and marked with red line. The Inc type in the figure showed the Inc type of *mcr-1.1*-harboring plasmid.

to colistin, which is considered as a last-line drug to treat multi-drug resistant (MDR) Gram-negative bacteria.

For the purpose to investigating genomic characteristics of *E. coli* RS1231S isolate, we performed NGS and revealed that this isolate belongs to ST131 O25:H4-fimH22 pandemic subclone and phylogroup B2.[20] ST131 belongs to the virulent phylogroup B2 and has been reported to induce a broad variety of infections, including meningitis, urinary tract infection, orchitis, urosepsis and peritonitis.[18,19] Besides, we found 22 virulence factors associated with adherent and toxin, indicating the high ability of adhesion and pathogenicity of *E. coli* RS1231S isolate, which is consistent with high virulence of ST131 *E. coli* pandemic population. However, the infection of *E. coli* RS1231S isolate was susceptible to the most antimicrobials and harbored rare acquired ARGs, which is not common since *E. coli* ST131 is considered the quintessential example of an international multidrug-resistant high-risk clone. The susceptibility of *E. coli* RS1231S isolate

means that there could be a good curative effect of antimicrobial treatment in this clinical case.

The plasmid-mediated colistin resistance gene *mcr-1.1* was firstly reported in animals in China, which poses a challenge for MDR Gram-negative bacteria treatment.[5] However, it is well investigated that food-producing animal is a reservoir of *mcr-1.1*.[5,23–28] Besides, a low prevalence of *mcr-1.1* among human infections was observed in several studies and barely reported in surgical site infections.[29–31] Collectively, we speculate that the *mcr-1.1*-harboring IncI2 plasmid (pRS1231-S-MCR-1.1) comes from animal sources, which is primarily supported by a high coverage and an identity of comparison with animal-related plasmid sequences and a remoted genetic relation of *E. coli* RS1231S from other sources. The lack of IS*Apl1* around *mcr-1.1* implying that the *mcr-1.1* was stable in the plasmid sequence and unlikely transfer through transposon.[21] Nevertheless, the high frequency of plasmid transfer of IncI2 plasmid needs to bring to
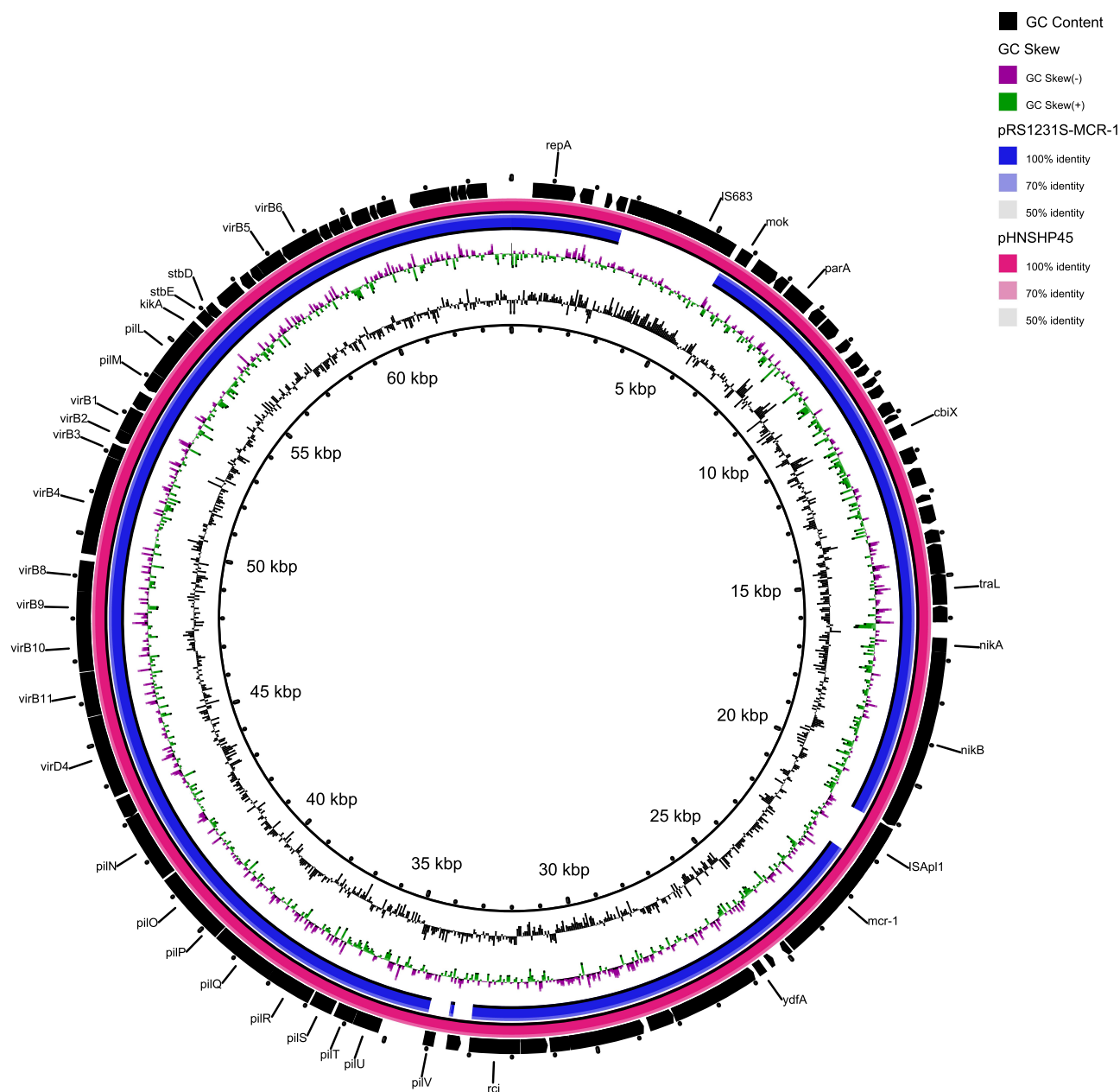
Figure 2 Plasmid comparison of pRS1231-S-MCR-1.1 and pHNSHP45 (Accession Number: KP347127.1). From the inner to outer ring: The inner ring represents the length scale. The second ring represents GC content. The third ring represents GC skew. The fourth ring with blue color represents the plasmid sequence of pRS1231-S-MCR-1.1. The fifth ring with pink color represents the sequence of pHNSHP45. The outer ring with black color represents the coding DNA sequences (CDSs) of pHNSHP45.

attention.[32] Even the incidence of infection by *mcr-1.1*-positive pathogen was rare, more surveillance should be implemented because the polymyxin was approved for the treatment of bacterial infections at the beginning of 2017 in China, which provided selective pressure in clinical infection communities. Since the development of new antimicrobials has been slow, the natural origins could be a new drug repository to explore.[33–35] Since the high prevalence and spreading of *mcr-1*-positive pathogens are attributed to the heavy use of colistin as a feed additive in animals, the Chinese government has been officially banned the use of colistin in feed animals. The latest research showed that the *mcr-1* prevalence has been dramatically decreased to a low prevalence.[36] The rational use of antimicrobials should be considered as a key measure to prevent the rising of antimicrobial resistance.

In conclusion, we reported the low incidence of postoperative infection of rhinoplasty was and a relatively not

severe AMR situation of these encountered pathogens. To the best of our knowledge, this is the first report of a rhinoplasty infection by *mcr-1.1*-positive *E. coli*. Future research should prospectively address the significance of AMR surveillance in the long term as well as the effectiveness of prophylactic measures and identify emerging new pathogen and resistance profile.

Ethics Statement

Written informed consent and ethics approval were exempted, since this retrospective study mainly focused on bacteria and patient intervention was not required. The patient data are confidential and compliance with the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest for this work and declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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