Trends in Antimicrobial Resistance of Coagulase-Negative Staphylococci in China From 2014 to 2023 and Characterization of a Rare Teicoplanin-Resistant *Staphylococcus haemolyticus*

Pan Chen [1], Yonghong Xiao [1,2]

1. Peking Union Medical College,Institute of Pathogen Biology,Chinese Academy of Medical Sciences, Beijing, 100730, China
2. State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, China-Singapore Belt and Road Joint Laboratory on Infection Research and Drug Development, National Medical Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine,Hangzhou, 310000, China

Background

Coagulase-negative staphylococci (CoNS) are common commensal organisms colonizing human skin and mucosal. CoNS are well recognized for their strong ability to form biofilms, which contributes to their frequent association with infections related to indwelling or implanted medical devices.

With the increasing use of invasive procedures and implantable devices, CoNS have emerged as major opportunistic pathogens in healthcare settings. Previous studies have demonstrated a continuous decline in CoNS susceptibility to most clinically used antimicrobial agents over recent decades. Due to their persistent colonization, biofilm-associated growth, and increasing antimicrobial resistance, CoNS infections pose substantial challenges for clinical management.

Antimicrobial resistance remains a critical issue in the treatment of CoNS infections. In particular, methicillin-resistant CoNS (MR-CoNS) have become highly prevalent worldwide, further limiting therapeutic options. Glycopeptide antibiotics, such as vancomycin and teicoplanin, exhibit good in vitro activity against MR-CoNS and are therefore commonly used as first-line agents for the treatment of these infections.

However, with the increased use of glycopeptides, changes in susceptibility among CoNS have drawn growing attention. In recent years, the phenomenon of “MIC creep” characterized by a gradual upward shift in minimum inhibitory concentration (MIC) values within the susceptible range, has been reported in *Staphylococcus aureus* treated with vancomycin. This trend may represent an early warning sign of reduced glycopeptide susceptibility and has been associated with an increased risk of therapeutic failure. In contrast, MIC creep in CoNS has been less frequently investigate.

Methods

1.Collection of Strains.

A total of CoNS isolates were collected from 2014 to 2023, across 54 hospitals in 21 provinces in China. The isolates were primarily recovered from blood samples and cerebrospinal fluid (CSF) specimens.

2.Antimicrobial Susceptibility.

Antimicrobial susceptibility was performed using broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute. The antibiotics tested included Penicillin, erythromycin, ciprofloxacin, clindamycin, rifampicin, oxacillin, ticoplanin and vancomycin.

3.Transmission Electron Microscopy (TEM).

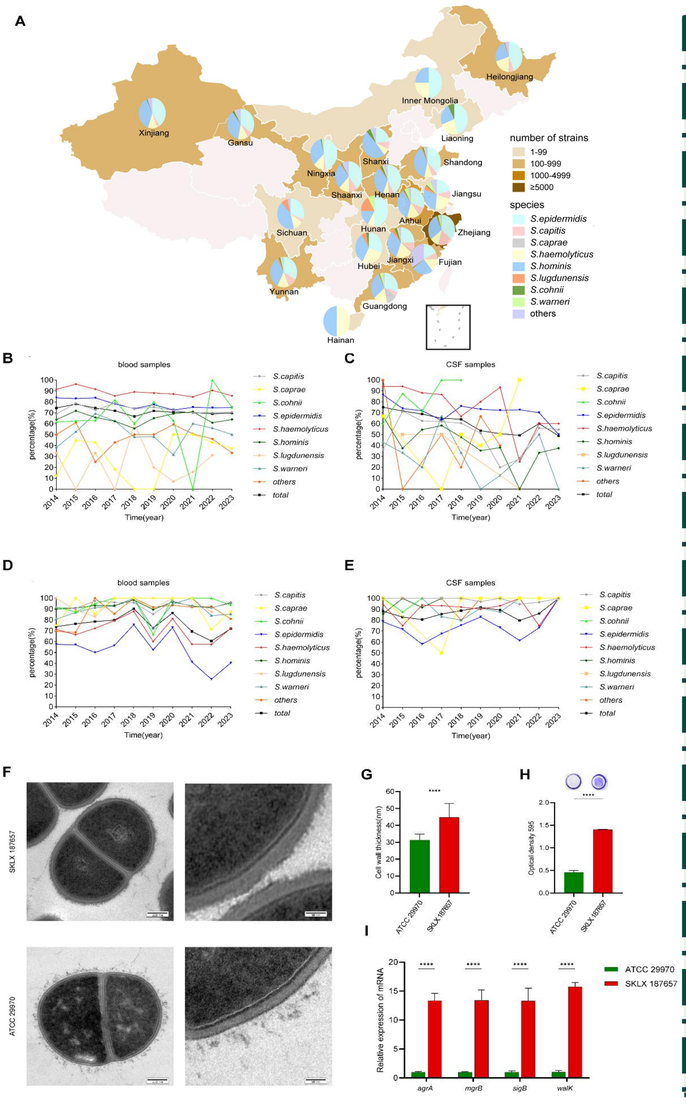
Bacteria were fixed with glutaraldehyde for 12 hours, followed by sequential treatment with osmium tetroxide, varying concentrations of ethanol, acetone, and embedding agent. Samples were observed under TEM and images were acquired for analysis.

4.Biofilm Detection.

Cultivate bacteria in liquid medium until the logarithmic growth phase is reached.Add the bacterial suspension to a 96-well plate and incubate for 24 hours. Remove the medium and wash three times with PBS buffer. Add an appropriate amount of crystal violet solution, incubate at room temperature for 30 minutes, and wash three times with PBS buffer.Dissolve crystal violet bound to the biofilm using acetic acid, then measure the absorbance at 600 nm using a spectrophotometer.

5.Detection of Gene Expression Levels.

Cultivate bacteria on a 37°C shaking incubator until the logarithmic growth phase. Extract RNA from bacteria using a commercial kit and reverse transcribe it into corresponding cDNA. Add cDNA, specific primers, enzyme-free water, and commercial pre-mix solution to a 96-well plate. Perform qPCR reactions using a real-time fluorescent quantitative PCR instrument and monitor fluorescence signals.



Results

A total of 11,277 CoNS isolates were collected from 54 hospitals across 21 provinces in China between 2014 and 2023 (Figure 1A). The isolates were obtained from blood and CSF specimens.

Among these, 10,253 isolates were recovered from blood samples. *Staphylococcus epidermidis* （S.*epidermidis*, n = 3,617) was the most frequently identified species, followed by *Staphylococcus hominis* (*S. hominis*, n = 3,333) and *Staphylococcus haemolyticus* (*S. haemolyticus*, n = 1,195).

A total of 1,024 isolates were obtained from CSF samples, showing a slightly different distribution pattern compared with blood isolates. *S. epidermidis* (n = 389) remained the predominant species; however, *Staphylococcus capitis* (n = 244) was more commonly detected than *S. haemolyticus*(n = 134) and *S. hominis* (n = 128). Notably, the species composition of CoNS varied across different provinces. In most regions, *S. epidermidis* was the dominant species, whereas *S. hominis* was the most frequently isolated species in provinces such as Anhui, Shandong, Jiangxi, Shanxi, and Jiangsu.

All isolates were tested for susceptibility to commonly used clinical antibiotics, including penicillin, erythromycin, ciprofloxacin, clindamycin, rifampin, oxacillin, teicoplanin, and vancomycin.

The resistance rates to oxacillin among CoNS isolates from blood (Figure 1B) and CSF (Figure 1C) sources were 69.81% and 48.78%, respectively. *S. haemolyticus* exhibited the lowest susceptibility to oxacillin, with resistance rates of 85.71% in blood samples and 60.00% in CSF samples. Over the 10-year period, the isolation rate of MRCoNS decreased over time in both blood-derived samples ($p<0.05$) and CSF-derived samples ($p<0.001$). This decline was primarily attributed to the decreased detection rates of MR-*S. epidermidis* in blood samples ($p<0.05$) and *S. haemolyticus* ($p<0.01$) and *S. hominis* ($p<0.05$) in CSF samples.

None of the strains included in this study exhibited resistance to vancomycin. Statistical analyses of isolates with vancomycin MIC values <2 mg/L were performed separately for blood isolates (Figure 1D) and CSF isolates (Figure 1E). The results demonstrated that vancomycin susceptibility among strains collected over the 10-year study period remained stable, with no evidence of a decline. Accordingly, the phenomenon of “MIC creep” was not observed. These findings indicate that CoNS isolates in China continue to maintain great and stable susceptibility to vancomycin.

Notably, a rare *S. haemolyticus* isolate resistant to teicoplanin (MIC = 32 μg/mL), designated SKLX 187657, was identified in this study. This strain was resistant to oxacillin but remained susceptible to vancomycin. Whole-genome sequencing of the strain revealed no vancomycin resistance-associated operons such as vanA or vanB.

Using the teicoplanin- susceptible standard strain *S. haemolyticus* ATCC 29970 as a control, TEM analysis of the ultrastructure of SKLX 187657 (Figure 1F) demonstrated a markedly thickened cell wall with a rough surface and irregular contours. The mean cell wall thickness of SKLX 187657 (44.71 ± 8.25 nm) was significantly higher than that of ATCC 29970 (31.33 ± 3.59 nm), with statistical significance (Figure 1G) ($t=8.140$, $df=58$, $p<0.0001$). Furthermore, compared to ATCC 29970, SKLX 187657 exhibited stronger biofilm formation capacity (Figure 1H) ($p<0.0001$).

To explore the mechanism underlying the thickened cell wall in SKLX 187657, the expression of genes associated with cell wall synthesis was further investigated.The key regulatory genes involved in cell wall synthesis—*agrA* ($p < 0.0001$), *mgrB* ($p < 0.0001$), *sigB* ($p < 0.0001$), and *walK* ($p < 0.0001$)—exhibit significantly higher expression levels in SKLX 187657 compared to ATCC 29970.

Conclusion

This 10-year nationwide study revealed dynamic changes in the species distribution and antimicrobial resistance patterns of CoNS isolates from bloodstream and cerebrospinal fluid specimens in China. MR-CoNS showed a declining trend over time, and vancomycin maintained stable and excellent activity, with no evidence of MIC creep. The identification of a rare teicoplanin-resistant *S. haemolyticus* isolate, characterized by cell wall thickening and upregulation of cell wall synthesis–related genes, highlights the potential for emerging glycopeptide resistance. Continuous surveillance and mechanistic studies remain essential to monitor and prevent the spread of such resistant strains in clinical settings.