ChinaRxiv

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201806.00223

Postprint: 5-Year Retrospective Analysis of In Vitro Antimicrobial Susceptibility of Ureaplasma spp. and Mycoplasma hominis and Quinolone Resistance Mechanisms

Authors: Liu Yali, Zhang Wenjuan, Wang Jie, Ye Sha 4, Zhao Ying, Wang He, Dou Hongtao, Guo Lina, Wang Yao, Hongli Sun, Liu Wenjing, Zhang Xiaojiang, Xie Xiuli, original English, Yang Qiwen, Xu Yingchun

Date: 2018-06-14T00:00:00+00:00

Abstract

Objective: To analyze the characteristics of in vitro culture and drug susceptibility of Ureaplasma species and Mycoplasma hominis over 5 consecutive years at Peking Union Medical College Hospital, and to investigate the mechanisms underlying quinolone resistance.

Methods: This study examined Ureaplasma species and Mycoplasma hominis isolates detected through in vitro culture over 5 consecutive years at Peking Union Medical College Hospital. By integrating patient and strain information, the characteristics of in vitro drug susceptibility were analyzed. Additionally, mutations in DNA gyrase (GyrA/GyrB) and topoisomerase IV (ParC/ParE) sequences were investigated in relation to quinolone resistance profiles.

Results: Overall, the susceptibility of Ureaplasma species combined with Mycoplasma hominis was significantly lower than that of either Ureaplasma species or Mycoplasma hominis alone. Except for macrolides, Ureaplasma species demonstrated lower susceptibility than Mycoplasma hominis to quinolones, tetracyclines, josamycin, pristinamycin, and doxycycline. Moreover, Ureaplasma species isolated from female patients exhibited lower in vitro susceptibility to azithromycin, erythromycin, clarithromycin, and ofloxacin compared to isolates from male patients. Ureaplasma parvum showed higher susceptibility rates to most antimicrobial agents than Ureaplasma urealyticum, particularly with a significantly higher susceptibility rate to tetracycline (difference of 25.8%), which was statistically significant ($p<0.05$). Furthermore, a total of 21 mutation sites were identified in GyrA, GyrB, ParC, and ParE sequences. Among these, the ParC S83L mutation predominated, accounting

for 96.22%. The ParC A136T and ParE R448K mutations were also detected, along with one case of combined GyrA L176F and ParC S83L mutation. Additionally, six novel mutation sites were identified (ParC L540F, R718W, Q767E, S789N, M828I, and I831T).

Conclusion: In vitro drug susceptibility is associated with infecting species, strain type, and patient gender. The resistance mechanism analysis revealed that alterations in the ParC amino acid sequence alone represent the primary resistance mechanism in this study, while the association of the newly discovered mutation sites with quinolone resistance requires further in-depth investigation.

Full Text

Prevalence and Antimicrobial Susceptibility of Ureaplasma and Mycoplasma hominis Over Five Consecutive Years and Mechanisms Responsible for Fluoroquinolone Resistance

Ya-Li Liu[1], Wen-Juan Zhang[2], Jie Wang[3], Sha Ye , Ying Zhao[1], He Wang[1], Hong-Tao Dou[1], Li-Na Guo[1], Yao Wang[1], Hong-Li Sun[1], Wen-Jing Liu[1], Xiao-Jiang Zhang[1], Xiu-Li Xie[1], Ying Yuan[1], Qi-Wen Yang[1], Ying-Chun Xu[1]

[1]Department of Clinical Laboratory, Peking Union Medical College Hospital, Beijing 100730, China
[2]Department of Clinical Laboratory, Beijing Youan Hospital of Capital Medical University/Hospital for Infectious Diseases of Baoding, Hebei, China
[3]Department of Clinical Laboratory, Hebei General Hospital, Hebei, China
Department of Clinical Laboratory, Bazhou People' s Hospital, Xinjiang, China

Corresponding author: Ying-Chun Xu, Email: xycpumch@139.com

Abstract

Objective: To analyze the culture and in vitro antimicrobial susceptibility characteristics of Ureaplasma and Mycoplasma hominis over five consecutive years at Peking Union Medical College Hospital and to investigate the mechanisms responsible for fluoroquinolone resistance.

Methods: This retrospective study reviewed all cases of Ureaplasma and Mycoplasma hominis detected by in vitro culture from September 2012 to April 2017 at Peking Union Medical College Hospital. Patient and species information were collected to analyze antimicrobial susceptibility patterns. Meanwhile, mutations in DNA gyrase (GyrA/GyrB) and topoisomerase IV (ParC/ParE) associated with fluoroquinolone resistance were detected.

Results: The in vitro susceptibility of Ureaplasma mixed with Mycoplasma hominis was significantly lower than that of either species alone. Except for

macrolides, Ureaplasma exhibited lower susceptibility than Mycoplasma hominis to quinolones, tetracyclines, josamycin, primycin, and doxycycline. Additionally, Ureaplasma isolates from female patients showed lower susceptibility to azithromycin, erythromycin, clarithromycin, and ofloxacin compared to those from male patients. Ureaplasma parvum demonstrated higher susceptibility rates to most antimicrobial agents than Ureaplasma urealyticum, particularly for tetracycline (25.8% difference, $p<0.05$). Furthermore, 21 mutation sites were identified in the sequences of GyrA, GyrB, ParC, and ParE. Among these, the ParC S83L mutation predominated at 96.22%, while ParC A136T and ParE R448K mutations were also detected, along with one case of combined GyrA L176F and ParC S83L mutation. Six novel mutation sites were discovered: ParC L540F, R718W, Q767E, S789N, M828I, and I831T.

Conclusion: In vitro antimicrobial susceptibility is associated with genus, species, and patient gender. The resistance mechanism analysis revealed that amino acid alterations solely in ParC represent the primary resistance mechanism in this study, while the relationship between the newly identified mutations and fluoroquinolone resistance requires further investigation.

Keywords: Ureaplasma; Mycoplasma hominis; Antimicrobial susceptibility; Resistance mechanisms

Introduction

Mycoplasmas and ureaplasmas belong to the class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae. To date, more than 118 Mycoplasma species and 7 Ureaplasma species have been identified, among which Mycoplasma pneumoniae, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma species are common human pathogens [1]. These organisms are mucosa-associated conditional pathogens that primarily colonize the mucosal surfaces of the lower respiratory and urogenital tracts, rarely penetrating the submucosal layers [1-2]. However, they may enter the bloodstream or other tissues and organs when host immunity is compromised. Currently, various detection methods are available, including DNA fluorochrome staining microscopy, antigen detection, serological tests, molecular biology assays, and culture. Culture-based methods are widely used in numerous domestic laboratories because they enable quantification and in vitro susceptibility testing [3], providing valuable guidance for clinical treatment. Based on this method, the present study retrospectively reviewed Ureaplasma and Mycoplasma hominis cases detected by in vitro culture at Peking Union Medical College Hospital between September 2012 and April 2017, examining their antimicrobial susceptibility profiles and preliminary investigations into fluoroquinolone resistance mechanisms.

Detection Methods

This study employed the Mycoplasma IST2 kit (bioMérieux, France), a colorimetric assay for Mycoplasma culture, identification, quantification, and antimicrobial susceptibility testing, with all procedures performed strictly according to the manufacturer' s instructions. Ureaplasma culture medium contains urea and the pH indicator phenol red. During growth, Ureaplasma hydrolyzes urea to produce ammonia, raising the medium pH and causing the phenol red indicator to change the medium color from yellow to red. Mycoplasma hominis metabolizes arginine to produce ammonia during growth, similarly elevating the medium pH and changing the color from yellow to red under the indicator' s influence.

Result Interpretation

For urea-arginine reagent vials, color changes were read at 24 and 48 hours of incubation. Yellow indicated negative results, while orange to red indicated positive results. Ureaplasma produced clear broth, whereas Mycoplasma hominis produced slightly flocculent turbid broth; turbid broth rendered results uninterpretable. For reaction cups, cup 4 (Uu 10) was read at 24 hours, and the remaining cups at 48 hours. Susceptibility breakpoints were defined as: tetracycline S 4 g/mL, ofloxacin S 1 g/mL R 4 g/mL; primycin S 2 g/mL.

Species Identification

Seventy-seven culture-positive clinical specimens were collected (only one specimen per patient). Nucleic acids were extracted directly using the QIAamp DNA MinElute Virus Spin Kit (QIAGEN GmbH, Hilden, Germany). Primers targeting the urease gene of Ureaplasma were designed: UF (5' -CGAAATTGTGATGAACGAAGG-3') and UR (5' -GGTGATAGCGTTAGATTAGGAG-3'). Conventional PCR was performed with the following conditions: initial denaturation at 94°C for 4 minutes, followed by 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The 418 bp PCR products were separated by 1% agarose gel electrophoresis, purified, and sequenced. DNA sequences were compared against two reference strains in the NCBI database: ATCC 27618 (serovar 8, Ureaplasma urealyticum type strain) and ATCC 27815 (serovar 3, Ureaplasma parvum type strain). Species identification required 100% sequence identity.

Resistance Mechanism Detection

Given the high resistance rates to fluoroquinolones (ciprofloxacin and ofloxacin), nucleic acids extracted from 70 specimens in Section 1.3 were further analyzed for resistance mechanisms. Based on studies by Beeton et al. [4-5], primers targeting the coding genes of GyrA, GyrB, ParC, and ParE enzymes were designed (Table 1). Conventional PCR amplification and sequencing were per-

formed. DNA sequencing results were translated into protein sequences and compared with reference sequences of Ureaplasma urealyticum ATCC 33699 (GenBank CP001184.1), Ureaplasma parvum serovar 1 ATCC 27813 (GenBank AF056983), serovar 3 ATCC 700970 (GenBank AF222894), serovar 6 ATCC 27818 (GenBank AF056984), and serovar 14 ATCC 33697 (GenBank AF056982) in the NCBI database to identify resistance-associated mutations.

Statistical Analysis

Differences in in vitro antimicrobial susceptibility rates were compared using Fisher' s exact test with =0.05; P<0.05 was considered statistically significant.

Results

Comparison of In Vitro Susceptibility Among Different Antimicrobial Agents

Among 694 positive specimens, 661 were Ureaplasma species, 12 were Mycoplasma hominis, and 21 were mixed Ureaplasma and Mycoplasma hominis infections. Analysis of in vitro susceptibility to nine commonly used antimicrobial agents (doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarithromycin, and primycin) revealed that primycin exhibited the strongest antimicrobial activity with a susceptibility rate of 99.0%, followed by doxycycline (98.1%), josamycin (95.8%), tetracycline (84.0%), clarithromycin (74.5%), azithromycin (43.5%), erythromycin (39.2%), ofloxacin (6.8%), and ciprofloxacin (2.9%) [Figure 1: see original paper].

Comparison of In Vitro Susceptibility Between Ureaplasma and Mycoplasma hominis

The results demonstrated that antimicrobial activity of the nine agents was species-dependent. Overall, the in vitro susceptibility of mixed Ureaplasma and Mycoplasma hominis infections was significantly lower than that of either species alone [Figure 2: see original paper]. Notably, josamycin susceptibility decreased by 44.7% compared to Ureaplasma alone and 47.6% compared to Mycoplasma hominis alone ($p<0.05$). Tetracycline susceptibility decreased by 22.5% versus Ureaplasma alone and 38.1% versus Mycoplasma hominis alone ($p<0.05$). Azithromycin susceptibility decreased by 44.8% versus Ureaplasma alone and 33.3% versus Mycoplasma hominis alone ($p<0.05$). Erythromycin susceptibility decreased by 40.4% versus Ureaplasma alone and 33.3% versus Mycoplasma hominis alone ($p<0.05$). Clarithromycin susceptibility decreased by 37.7% versus Ureaplasma alone ($p<0.05$) and 20.2% versus Mycoplasma hominis alone ($p>0.05$). Doxycycline susceptibility decreased by 17.6% versus Ureaplasma alone and 19.0% versus Mycoplasma hominis alone ($p>0.05$). Primycin susceptibility decreased by 3.9% versus Ureaplasma alone and 4.8%

versus Mycoplasma hominis alone ($p>0.05$). Ofloxacin susceptibility decreased by 6.7% versus Ureaplasma alone and 16.7% versus Mycoplasma hominis alone ($p>0.05$). Ciprofloxacin susceptibility decreased by 2.7% versus Ureaplasma alone and 8.3% versus Mycoplasma hominis alone ($p>0.05$) [Figure 2: see original paper].

Additionally, Ureaplasma exhibited slightly lower susceptibility than Mycoplasma hominis (except for macrolides), though these differences were not statistically significant [Figure 2: see original paper].

Comparison of In Vitro Susceptibility of Ureaplasma Between Genders

The study also compared Ureaplasma susceptibility between genders . Ureaplasma isolates from female patients showed significantly lower susceptibility to all nine antimicrobial agents compared to those from male patients, particularly for macrolides (erythromycin, azithromycin, clarithromycin) and ofloxacin. Specifically, compared to male patients, susceptibility rates in female patients decreased by 22.0% for erythromycin ($p<0.05$), 20.2% for azithromycin ($p<0.05$), 9.5% for clarithromycin ($p<0.05$), and 6.9% for ofloxacin ($p<0.05$). No significant differences were observed for doxycycline, josamycin, primycin, or tetracycline ($p>0.05$).

Comparison of In Vitro Susceptibility Between Ureaplasma parvum and Ureaplasma urealyticum

Since the culture method cannot differentiate Ureaplasma species, and considering potential susceptibility differences between species, 77 non-duplicate Ureaplasma-positive specimens collected within the past year were subjected to PCR and sequencing analysis. Among these, 66 were identified as Ureaplasma parvum and 11 as Ureaplasma urealyticum. Due to the small number of Ureaplasma urealyticum isolates, Fisher' s exact test was used to compare susceptibility between the two species . Ureaplasma parvum demonstrated higher susceptibility rates to most antimicrobial agents than Ureaplasma urealyticum, with a significantly higher susceptibility to tetracycline (25.8% difference, $p<0.05$).

Screening of Fluoroquinolone Resistance-Related Mutations

Given the high resistance rates to fluoroquinolones (ciprofloxacin and ofloxacin), nucleic acids from 70 culture-positive clinical specimens (7 of the 77 positive specimens had insufficient volume for this analysis) were examined. Primers targeting the coding genes of GyrA, GyrB, ParC, and ParE enzymes were designed, and after amplification and sequencing, the translated protein sequences were compared with reference sequences. Multiple mutation sites were identified, predominantly in the ParC amino acid sequence. No mutations were detected in GyrB, while single mutations were found in GyrA and ParE .

Among the 70 specimens, 61 (87.14%) were resistant to ciprofloxacin and/or ofloxacin, while 9 (12.86%) were intermediate and/or susceptible. Of the 61 resistant specimens, 53 (86.89%) harbored mutations. The ParC S83L substitution (serine to leucine at position 83) was most frequent, occurring in 51 isolates (96.22%). Other detected mutations included ParC A136T (1 isolate, strain 73), ParC M105I (1 isolate, strain 34), ParC D530N (4 isolates, strains 32/42/73/76), ParC L553I (3 isolates, strains 32/42/76), ParC T570A (4 isolates, strains 32/42/73/76), ParC I702V (4 isolates, strains 32/42/73/76), ParC R718W (2 isolates, strains 71 and urine 28), ParC A734T (1 isolate, strain 73), ParC A735S (4 isolates, strains 32/42/73/76), ParC I743M (4 isolates, strains 32/42/73/76), ParC Q767E (1 isolate, strain 73), ParC S789N (4 isolates, strains 32/42/73/76), ParC I790M (4 isolates, strains 32/42/73/76), ParC D794N (4 isolates, strains 32/42/73/76), ParC D799N (4 isolates, strains 32/42/73/76), ParC M828I (1 isolate, strain 73), and ParC I831T (4 isolates, strains 32/42/73/76).

Additionally, a single mutation was identified in GyrA: L176F (leucine to phenylalanine at position 176, 1 isolate, strain 86). A single mutation was also found in ParE: R448K (arginine to lysine at position 448, 1 isolate, strain 81). Among the 9 intermediate and/or susceptible specimens, 2 harbored mutations: ParC L540F (1 isolate, strain 99) and a combination of ParC D530N, T570A, A735S, I743M, Q767E, S789N, I790M, D794N, D799N, and I831T (1 isolate, strain 27).

Discussion

Current treatment of Ureaplasma infections primarily involves tetracyclines, macrolides, and fluoroquinolones. However, antimicrobial resistance has become increasingly severe with rising fluoroquinolone resistance rates. The culture method offers the advantage of quantification while simultaneously determining in vitro susceptibility to nine antimicrobial agents, providing crucial information for clinical management. Our findings demonstrate that in vitro susceptibility varies according to species, strain, and patient gender. For instance, significant differences exist between Ureaplasma and Mycoplasma hominis susceptibility profiles. Overall, mixed Ureaplasma and Mycoplasma hominis infections exhibited significantly lower susceptibility than either species alone, particularly for josamycin, tetracycline, azithromycin, and erythromycin ($p<0.05$). Due to intrinsic macrolide resistance in Mycoplasma hominis, Ureaplasma showed significantly higher susceptibility to macrolides, while demonstrating lower susceptibility to quinolones, tetracyclines, josamycin, primycin, and doxycycline. Although not statistically significant, these trends align with domestic and international data [3,6-7].

Furthermore, our study revealed significant gender-related differences in susceptibility. Ureaplasma isolates from female patients showed markedly lower susceptibility to azithromycin, erythromycin, clarithromycin, and ofloxacin compared to male patients ($p<0.05$). Previous studies have reported a higher prevalence

of infections in female patients [3,8-9], and we speculate that antimicrobial usage during treatment may contribute to increased resistance, though additional data are needed to support this hypothesis.

Given the high fluoroquinolone resistance rates (>80%), we screened for common resistance mechanisms. The key targets of fluoroquinolones are DNA gyrase and topoisomerase IV, encoded by gyrA/gyrB and parC/parE genes, respectively. Alterations in GyrA, GyrB, ParC, and ParE sequences directly confer fluoroquinolone resistance, with changes in the quinolone resistance-determining regions (QRDR) of GyrA and ParC being most critical. The QRDR of GyrA spans nucleotides 202-531 at the 5' end (amino acid residues 68-177), while the QRDR of ParC spans nucleotides 152-456 at the 3' end (amino acid residues 51-152). Our primers covered these QRDR regions, and after amplification, sequencing, and translation, sequences were compared with multiple reference sequences (detailed in Materials and Methods). We identified 21 mutation sites, including previously reported ParC S83L and A136T mutations and ParE R448K mutation [4-5,10-18]. Other reported mutations included ParC M105I, D530N, L553I, T570A, I702V, A734T, A735S, I743M, I790M, D794N, and D799N [11,13-14], though their direct contribution to fluoroquinolone resistance remains unclear. We also discovered six novel mutations: ParC L540F, R718W, Q767E, S789N, M828I, and I831T, which are reported here for the first time and require further investigation to determine their role in fluoroquinolone resistance.

Notably, the ParC S83L mutation alone accounted for 94.34% (50/53) of resistant isolates, with only one case showing combined GyrA L176F and ParC S83L mutations. In contrast, a meta-analysis of 14 studies from 2000-2012 found that combined GyrA and ParC mutations were common internationally (47.37%, 72/152), followed by GyrA mutations alone (28.29%, 43/152, most commonly D112E) and ParC mutations alone (23.68%, 36/152, most commonly S83L) [19]. However, studies from 2013 onward from the United States, Japan, New Zealand, and China have predominantly reported ParC S83L as the sole mutation [14-18,20], suggesting potential associations with species, serovar differences, and evolution. This trend also indicates that contemporary circulating strains exhibit higher resistance rates than historical isolates, as ParC mutations alone confer greater fluoroquinolone resistance (particularly to ciprofloxacin) compared to combined GyrA and ParC mutations. Additionally, several resistant isolates lacked identifiable mutations, suggesting alternative resistance mechanisms such as resistance plasmids, efflux pumps, altered membrane permeability, or hydrolytic enzymes [21] that warrant further investigation.

References

[1] Jorgensen JH, Pfaller MA. Manual of Clinical Microbiology (11th Edition) [S]. 2015.
[2] Garcia LS. Clinical Microbiology Procedures Handbook (Third Edition) [S].

2007.
[3] Zeng XY, Xin N, Tong XN, et al. Prevalence and antibiotic susceptibility of Ureaplasma urealyticum and Mycoplasma hominis in Xi' an, China [J]. Eur J Clin Microbiol Infect Dis, 2016, 35:1941-1947.
[4] Beeton ML, Chalker VJ, Kotecha S, et al. Comparison of full gyrA, gyrB, parC and parE gene sequences between all Ureaplasma parvum and Ureaplasma urealyticum serovars to separate true fluoroquinolone antibiotic resistance mutations from non-resistance polymorphism [J]. J Antimicrob Chemother, 2009, 64:529-538.
[5] Beeton ML, Chalker VJ, Maxwell NC, et al. Concurrent titration and determination of antibiotic resistance in ureaplasma species with identification of novel point mutations in genes associated with resistance. Antimicrob Agents Chemother, 2009, 53:2020-2027.
[6] Lee MY, Kim MH, Lee WI, et al. Prevalence and Antibiotic Susceptibility of Mycoplasma hominis and Ureaplasma urealyticum in Pregnant Women [J]. Yonsei Med J, 2016, 57(5):1271-1275.
[7] Redelinghuys MJ, Ehlers MM, Dreyer AW, et al. Antimicrobial susceptibility patterns of Ureaplasma species and Mycoplasma hominis in pregnant women [J]. BMC Infect Dis, 2014, 14:171.
[8] Zhang R, Ye AL, Kong LJ, et al. Molecular biological detection and analysis of three sexually transmitted diseases in clinical patients [J]. Modern Laboratory Medicine, 2015, 30(3): 107-110.
[9] Song T, Ye A, Xie X, et al. Epidemiological investigation and antimicrobial susceptibility analysis of Ureaplasma species and Mycoplasma hominis in outpatients with genital manifestations [J]. J Clin Pathol, 2014, 67(9):817-820.
[10] Piccinelli G, Gargiulo F, Biscaro V, et al. Analysis of mutations in DNA gyrase and topoisomerase IV of Ureaplasma urealyticum and Ureaplasma parvum serovars resistant to fluoroquinolones. Infect Genet Evol, 2017, 47:64-67.
[11] Xiao L, Crabb DM, Duffy LB, et al. Chromosomal mutations responsible for fluoroquinolone resistance in Ureaplasma species in the United States. Antimicrob Agents Chemother, 2012, 56(5):2780-2783.
[12] Bébéar CM, Renaudin H, Charron A, et al. DNA gyrase and topoisomerase IV mutations in clinical isolates of Ureaplasma spp. and Mycoplasma hominis resistant to fluoroquinolones. Antimicrob Agents Chemother, 2003,47(10):3323-3325.
[13] Duffy L, Glass J, Hall G, et al. Fluoroquinolone resistance in Ureaplasma parvum in the United States. J Clin Microbiol, 2006, 44(4):1590-1591.
[14] Song J, Qiao Y, Kong Y, et al.Frequent topoisomerase IV mutations associated with fluoroquinolone resistance in Ureaplasma species.J Med Microbiol, 2015, 64(11):1315-1320.
[15] Fernández J, Karau MJ, Cunningham SA, et al.Antimicrobial Susceptibility and Clonality of Clinical Ureaplasma Isolates in the United States. Antimicrob Agents Chemother, 2016, 60(8):
[16] Kamiya Y, Shimada Y, Ito S, et al. Analysis of the quinolone-resistance determining region of the gyrA gene and the analogous region of the parC

gene in Ureaplasma parvum and Ureaplasma urealyticum detected in first-void urine of men with non-gonococcal urethritis. J Antimicrob Chemother, 2013, 68(2):480-482.
[17] Kawai Y, Nakura Y, Wakimoto T, et al. In vitro activity of five quinolones and analysis of the quinolone resistance-determining regions of gyrA, gyrB, parC, and parE in Ureaplasma parvum and Ureaplasma urealyticum clinical isolates from perinatal patients in Japan. Antimicrob Agents Chemother, 2015, 59(4):2358-2364.
[18] Schneider SC, Tinguely R, Droz S, et al. Antibiotic Susceptibility and Sequence Type Distribution of Ureaplasma Species Isolated from Genital Samples in Switzerland. Antimicrob Agents Chemother, 2015, 59(10):6026-6031.
[19] Zhu XF, Peng HX, Li M. Systematic review of quinolone resistance-determining region mutations inducing fluoroquinolone resistance in Ureaplasma urealyticum. Journal of Clinical Laboratory Science, 2014, 32(12): 46-48.
[20] Jin H, Qi C, Zou Y, et al. Biochanin A partially restores the activity of ofloxacin and ciprofloxacin against topoisomerase IV mutation-associated fluoroquinolone-resistant Ureaplasma species.J Med Microbiol, 2017, 66(11):1545-1553.
[21] Robicsek, AJ. Strahilevitz, GA, Jacoby, M, et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med, 2006, 12:83-88.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.