Biological Characteristics of Artificially Induced Drug Resistant Staphylococcus Aureus

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Abstract: In order to compare the biological characteristics of artificially induced Staphylococcus aureus and primary sensitive Staphylococcus strains, and to explore the resistance mechanism of the artificially induced Staphylococcus aureus. Staphylococcus was used as a test strain. According to NCCLS recommended paper diffusion method, the drug sensitivity test was used to compare the original Staphylococcus and 12 species of Staphylococcus aureus artificially induced by the original sensitive Staphylococcus. The sensitivity of antibiotics. Biochemical tests were used to compare the biochemical characteristics of primary and resistant strains. The 16SrRNA gene and the mecA gene (drug resistance gene) of the primary strain and the resistant strain were amplified by PCR, and the 16S rRNA gene and the mecA gene of the primary strain and the resistant strain were compared. Results Primary Staphylococcus was sensitive or even extremely sensitive to 12 antibiotics. Although resistant to Staphylococcus was completely resistant to β-lactam antibiotics in these 12 antibiotics, it was resistant to other non-β-lactam antibiotics (sugars). The sensitivity of peptides, quinolones, tetracyclines, aminoglycosides is increased. Among them, the sensitivity of aminoglycosides represented by neomycin and glycopeptides represented by vancomycin is particularly high. The results of biochemical tests showed that the ability of Staphylococcus aureus to ferment mannitol was weaker than that of primary bacteria, and it could not ferment mannitol within 24 h and mannitol in 48 h. PCR results showed that both the original Staphylococcus and the resistant strains could amplify the same 16SrRNA gene, but they could not amplify the mecA gene, indicating that the 16SrRNA gene did not mutate, and the primary and resistant bacteria did not contain mecA gene. The relationship between this series of changes in drug-resistant strains and drug resistance still needs further study. Conclusion: When a bacterium is resistant to a certain drug, its sensitivity to other drugs will change accordingly.

1. Introduction

"Super bacteria" is one of the hot topics in the society. The acquisition of bacterial resistance is generally considered to be that the sensitivity of the drug is reduced or even disappeared after the bacteria repeatedly contact with the drug. In real life, the use of antibiotics has not been well regulated, making drug-resistant bacteria appear one after another. But in the past two decades, humans have not found any new antibiotics to deal with bacteria. Therefore, the research on drug resistance mechanism of drug-resistant bacteria is urgent. Among staphylococcus, Staphylococcus aureus is the main pathogen of purulent infection in humans and animals, which can cause bacterial food poisoning, pneumonia, pericarditis and meningitis, and even systemic infections such as sepsis and sepsis [1]. With the large-scale and extensive use of antibiotics, the infection of the bacteria has been increasing year by year, and the drug resistance rate has increased year by year. The drug resistance and virulence resistance to methicillin-resistant Staphylococcus aureus MRSA have become widespread. Distributed in the environment of hospitals and communities, there are great difficulties in treatment, which has caused great clinical attention [2]. According to the current research, bacterial resistance mechanisms are diverse, such as the super drug resistance gene

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NDM-1. In this paper, the biological exploration of Staphylococcus which is artificially induced by antibiotics under laboratory conditions is explored, and it is hoped that the relationship between drug resistance and related genes or apparent phenomena can be found.

2. Materials and Methods

2.1 Source and Identification of Bacteria

The primary sensitive Staphylococcus strain was provided by the Guangdong Provincial Key Laboratory of Preventive Veterinary Medicine of Foshan University of Science and Technology. It was isolated and purified by plate scribing. The purified strain was inoculated into high-salt mannitol medium and cultured at 37 °C for 24 h. The morphological characteristics of the colonies, Gram stain microscopy, and the characteristics are consistent with Staphylococcus. The purified strain was stored in a refrigerator at -20 °C with 50% glycerol. Staphylococcus aureus is artificially induced by cefazolin from primary sensitive staphylococci and stored in the laboratory.

2.2 Reagents and Instruments

The drug sensitive papers of 12 antibiotics were purchased from Hangzhou Microbial Reagent Co., Ltd., and the batch numbers were: cefazolin (batch number: S1012), ceftazidime (batch number: S1019), penicillin (batch number: S1001), oxacillin (batch number: S1002), ampicillin (batch number: S1003), vancomycin (batch number: S1054), ofloxacin (batch number: S1049), norfloxacin (batch number: S1047), enrofloxacin (batch number: S1081), Neomycin (batch number: S1034), tetracycline (batch number: S1036), doxycycline (batch number: S1037); cefazolin standard (batch number: 0421-9603) purchased from Guangdong Provincial Institute of Drug Control, titer 99.3 % According to the published mecA sequence and 16SrRNA sequence, the upstream and downstream primers for the mecA gene were synthesized by Bioengineering (Shanghai) Co., Ltd., M1 1282-5'-AAAATCGATGGTAAAGGTTGGC-3'-1303, M2 1739-5' -AGTTCTGCAGTACCGGATTTGC-3'-1814; PCR amplification product is 533 bp; and DNA sequence primer sequence for synthesis of 16SrRNA: upstream primer -5'-GTGCACATCTTGACGGTACC-3', downstream primer -5'-CGAAGGGGAAGGCTCTATC-3'. The PCR amplification sequence is 565 bp; the PCR amplification instrument model is BIO-RAD-T100; common nutrient broth, nutrient agar, mannitol NaCl nutrient agar, and glucose, lactose, maltose, mannitol, and sucrose biochemical identification tubes are purchased from Guangdong Huankai Microbiology Technology Co., Ltd. The reagents are used within the validity period.

2.3 Test Methods

The sensitivity of the two to 12 antibiotics was compared by the NCPLS recommended paper diffusion method (K-B method). After purification of the original strain and the resistant strain on the common agar plate, a single colony was inoculated to the liquid medium for 12 h, and the concentration of the broth was adjusted to 1.5×108 CFU/mL. The prepared bacterial liquid was extracted with a sterile cotton swab and uniformly coated on the surface of the agar plate. After drying, the drug-sensitive paper is evenly applied to the surface of the bacteria-containing agar plate, cultured in a 37 ° C incubator for 18 to 24 hours, and the inhibition zone is measured, according to the 2017 and 2013 American Committee for Clinical and Experimental Standards (CLSI). The standard interpretation results [3, 4] are compared.

The primary strain and the resistant strain were tested for sugar fermentation to compare the biochemical characteristics of the two strains. Single colonies of primary and resistant strains were picked to biochemical fermentation tubes of glucose, lactose, maltose, mannitol and sucrose. The cells were cultured for 18-24 hours, and their color changes were observed to compare biochemical characteristics.

The original strain and the DNA of the resistant strain were extracted using the modified SDS method [5]. Take 1 mL of the bacterial solution in a 1.5 mL sterile EP tube, centrifuge at 10,000

r•min-1 for 5 min, and discard the supernatant. Add 150 μL of sterile deionized water, 15 μL of 10% SDS, 50 μL of 5 mol•L-1 NaCl, and incubate at 65 °C for 10 min. Add an equal volume (215 μL) of phenol/chloroform/isoamyl alcohol (25:24:1) and mix at 10,000 r•min-1 for 5 min. The supernatant was transferred to a new tube, and an equal volume of chloroform/isoamyl alcohol (24:1) was added to the supernatant, mixed, and centrifuged at 10,000 r•min-1 for 5 min. The supernatant was transferred to a new tube, and the supernatant was mixed with 0.6 volumes of isopropanol, allowed to stand for 10 min, and centrifuged at 10,000 r•min-1 for 5 min. Discard the supernatant, add 200 μL of 70% ethanol, centrifuge at 12000 r•min-1 for 2 min, and discard the supernatant. After repeated washing, resuspend the DNA in 60 μL of double distilled water and store at -20 °C.

2 μL DNA template was added to the PCR reaction system, primer 1, 0.5 μL, primer 2, 0.5 μL, 9.5 μL of non-ionized water, 12.5 μL of Premix, total volume of 25 μL, and mixed by transient centrifugation and placed in a PCR instrument. Control: 95 °C 5 min pre-denaturation, 95 °C 30 s, 44 °C 30 s, 72 °C 30 s, 35 cycles, 72 °C, extension 8 min. The primary strain and the resistant strain simultaneously amplified the 16SrRNA gene, and the 16SrRNA gene was amplified positive, but not amplified. 4 μL of PCR product was placed into a 1.5 g/100 mL agarose gel, 1×TAE was used as a running buffer, DNA Marker D (100-2000 bp) was used, and electrophoresis was performed by horizontal electrophoresis. The voltage was 120 V. At 20 min, the results were observed outside the 300 nm purple light, and the gel imaging system was imaged and recorded.

2 μL DNA template was added to the PCR reaction system, upstream primer, 0.5 μL, downstream primer, 0.5 μL, 9.5 μL of deionized water, 12.5 μL of Premix, total volume of 25 μL, and mixed by transient centrifugation and placed in a PCR instrument: 94 °C 10 min pre-denaturation, 94 °C 30 s, 54 °C 45 s, 72 °C 45 s cycle 30 times, 72 °C extension 10 min. The primary strain and the resistant strain simultaneously amplified the mecA gene, and the mecA gene was amplified positive, but not amplified. 5 μL of PCR product was placed into a 1.5 g/100 mL agarose gel, 1×TAE was used as a running buffer, DNA Marker D (100-2000 bp) was used, and electrophoresis was performed in a horizontal electrophoresis tank at a voltage of 80 V for 1 h. The 300 nm UV lamp was observed and the gel imaging system was imaged and recorded.

3. Results and Analysis

Table.1 Data records of inhibition zone of primary and resistant strains and interpretation criteria of 12 antibiotic inhibition zones

Drug name (μg)	dose	Inhibition zone diameter(mm)		Drug inhibition zone interpretation standard(mm)		
		Primary strain	Drug resistant strain	Drug resistance	intermediary	sensitive
penicillin	10	38	9	≤28	-	≥29
Oxacillin	1	24	10	≤10	11～12	≥13
Ampicillin	10	36	9	≤28	-	≥29
Cefazolin	30	39	9	≤14	15～17	≥18
Ceftazidime	30	20	6	≤14	15～17	≥18
Vancomycin	30	16	23	-	-	≥15
Ofloxacin	5	30	35	≤12	13～15	≥16
Norfloxacin	10	24	30	≤12	13～16	≥17
Enrofloxacin	5	26	35	≤15	16～20	≥21
tetracycline	30	26	35	≤14	15～18	≥19
Doxycycline	30	21	30	≤12	13～15	≥16
Neomycin	30	19	33	≤12	13～16	≥17

Note: Drug inhibition zone interpretation standard data source NCCLS-2017, neomycin and doxycycline inhibition zone data source NCCLS-2013

Comparison of sensitivity of primary sensitive Staphylococcus and Staphylococcus aureus to 12 antibiotics. The results of drug susceptibility test showed that the primary strains were sensitive to 12 antibiotics, and the artificially induced drug-resistant strains were completely resistant to

β-lactam antibiotics represented by cefazolin, and their inhibition zones were significantly reduced. At the same time, we found that the sensitivity of Staphylococcus aureus to non-β-lactam antibiotics changed, generally showing increased sensitivity, see Table 1.

Results of biochemical characteristics of primary sensitive Staphylococcus and Staphylococcus aureus. The results of biochemical reaction showed that the primary strain fermented glucose, lactose, sucrose, mannitol and semi-fermented maltose for 24 h; the artificially induced drug-resistant strain fermented glucose, lactose, sucrose, semi-fermented maltose for 24 h, and did not ferment mannitol for 48 h. Semi-fermented mannitol, see Table 2.

Table.2 Biochemical reaction records of primary and resistant bacteria

	Glucose fermentation	Lactose fermentation	Maltose fermentation	Mannitol fermentation	Sucrose fermentation
Primary strain	+	+	Semifermentation	+	+
Drug resistant strain	+	+	Semifermentation	*	+

*: 24 h without fermentation, 48 h semi-fermentation

The primary strain and the resistant strain were amplified according to the above 16SrRNA gene fragment primers, and the bands between 500 and 750 bp were amplified, which were consistent with the theoretical values. It can be proved that both the primary strain and the resistant strain are Staphylococcus, and the 16SrRNA gene does not mutate. The amplification results are shown on the left.

The primary strain and the resistant strain did not amplify the mecA gene based on the above mecA gene fragment primers. This result indicates that the resistance mechanism of artificially induced Staphylococcus aureus is not related to the mecA resistance gene. The amplification results are shown on the right.

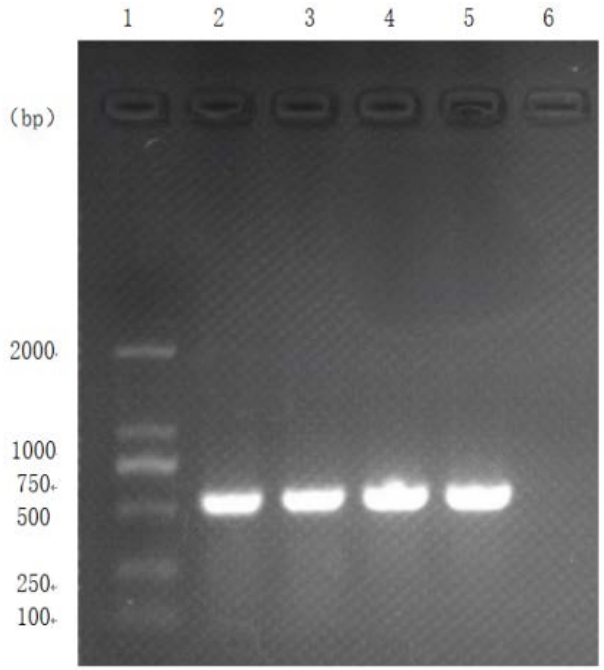

Fig 1 PCR electrophoresis map of 16SrRNA gene fragment

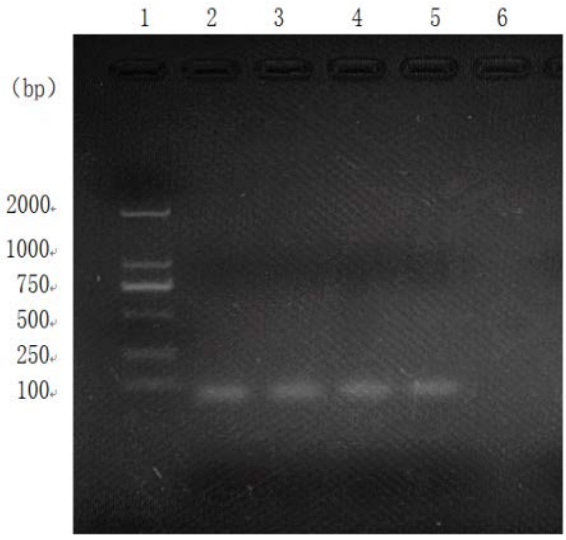

Fig 2 PCR electrophoresis results of primary strain and resistant strain mecA gene

4. Discussion

In this experiment, the apparent phenomenon showed that the drug-resistant Staphylococcus aureus artificially induced by cefazolin showed complete resistance to the tested β-lactam antibiotics and was sensitive to the tested non-β-lactam antibiotics. The degree has increased. Cefazolin is a beta-lactam antibiotic and is the first generation cephalosporin. The antibacterial mechanism of β-lactam antibiotics is the synthesis of cell walls of bacteriostatic bacteria. The cephalosporins all contain a β-lactam ring, and the mechanism of action is to inhibit the bacterial cell wall synthesis by binding to the penicillin binding protein on the bacterial cell wall, resulting in bacterial death. [8] According to the results of this experiment, the conserved gene 16SrRNA of Staphylococcus aureus did not mutate, and the drug resistance of the drug-resistant strain may be independent of 16SrRNA; at the same time, the primary strain and the resistant strain did not expand. The addition of the mecA resistance gene indicates that the resistance mechanism of the drug resistance is also not directly related to the mecA gene.

There are three mechanisms for the resistance of staphylococci to β-lactam antibiotics: 1. Staphylococci produce β-lactamase capable of hydrolyzing β-lactam drugs to inactivate drugs, including broad-spectrum enzymes. , broad-spectrum enzyme β-lactamase (ESBL), metalloenzyme, AmpC enzyme, etc.; 2. The target of staphylococcal and β-lactam drugs changes, so that the drug can not bind to bacteria: β-lactam Antibiotics must be combined with target sites on the bacterial cell membrane to exert bactericidal action. According to the decreasing molecular weight or the increasing migration speed, the bacterial membrane protein-penicillin binding protein (PBP) is divided into PBP1, PBP2, PBP3 and PBP4. , PBP5, PBP6, etc. Different lactam antibiotics bind to their corresponding PBP and inhibit the cell wall biosynthesis of bacteria, which can cause the death of the bacteria and thus achieve the sterilization effect. Once the antibiotic-acting PBP changes, affecting the binding affinity, it will cause bacterial resistance; 3. The permeability of the drug membrane outer membrane changes, the drug can not pass the bacterial membrane: the bacterial cell membrane is a kind of height A selectively permeable barrier that controls the exchange of substances inside and outside the cell. The permeability barrier of most membranes has a lipid bilayer structure that allows the passage of lipophilic drugs with a channel protein embedded in the lipid bilayer. A non-specific, water-soluble diffusion channel that spans the cell membrane. If the bacterial channel protein is lost or reduced, it will also cause drug resistance. [8]

The experimental results show that the drug-resistant strain has increased sensitivity to other antibiotics (glycopeptides, quinolones, tetracyclines, aminoglycosides). The inhibition zones of the quinolone antibiotics ofloxacin, norfloxacin and enrofloxacin increased from 30, 24, 26 mm to 35,

30, 35 mm, respectively. The antibacterial properties of the tetracycline antibiotic tetracycline and doxycycline The circle increased from 26, 21 mm to 35, 30 mm, the inhibition zone of the aminoglycoside antibiotic neomycin increased from 19 mm to 33 mm, and the inhibition zone of the glycopeptide antibiotic vancomycin increased from 16 mm to 23 mm. . Among them, the inhibition zone of neomycin increased the most, and the sensitivity increased most obviously, followed by enrofloxacin and tetracycline. Combined with the above-mentioned drug resistance mechanism, we speculate that the drug-resistant bacteria change the permeability of the outer membrane of the cell membrane, so that the drug can not pass the bacterial cell membrane to produce resistance to β-lactam drugs, but due to the drug-resistant bacteria The permeability of the cell membrane changes, and it is likely to increase the permeability of other antibiotics, making the strain more sensitive to non-β-lactam antibiotics. Research on this mechanism is being further explored.

In addition, the results of biochemical tests showed that the drug-resistant staphylococci significantly reduced the decomposition and fermentation capacity of mannitol, did not ferment mannitol within 24 h, and only semi-fermented mannitol for 48 h. At this point, there are still few current studies, and there are no relevant theories or articles to explain this change. Therefore, in the follow-up experiments, it will be further explored whether the weakened ability of the resistant Staphylococcus aureus to ferment mannitol is related to the development of drug resistance. To clarify the relationship between all types of drug resistance mechanisms and biological characteristics, it can provide a good reference for clinical treatment of drug-resistant staphylococci.

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