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Distribution and drug resistance analysis of clinically isolated pathogenic bacteria in a tertiary hospital from 2018 to 2020

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Abstract: **Objective:** To investigate the distribution and drug resistance of clinically isolated pathogens in the Second Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine during 2018-2020, so as to provide reference for rational clinical use of antimicrobial agents. **Methods**: Biological bacteria identification/susceptibility analysis system and the time of flight mass spectrometer identification of bacteria, with reference to the clinical laboratory standardization committee interpretation standard drug susceptibility of 2020 results, application of disc diffusion method (K-B) bacteriostatic circle diameter, the measurement of antimicrobial agents susceptibility results interpretation, WHONET5.6 statistical analysis software in our separation rate of bacteria and drug resistance. **Results:** In 3 years, a total of 3586 strains of pathogenic bacteria were detected (excluding the duplicative strains from the same site in the same patient), 1582 strains of *Gram-positive bacteria* (44.1%), 1542 strains of *Gram-negative bacteria* (43.0%), and 462 strains of *fungi* (12.9%). *Gram-negative bacteria* were mainly *Escherichia coli* (547 strains,35.5%) and *Klebsiella pneumoniae* (256 strains,16.6%). *ESBLs* producing bacteria of *Escherichia coli* accounted for 60.0%, and acid producing *Klebsiella* of producing *ESBLs* (40.7%) was higher than *klebsiella pneumoniae* (29.3%). The detection rate of carbapenem-resistant acid-producing *Klebsiella* (5.5%) was higher than that of *Klebsiella pneumoniae* (2.3%) and *Escherichia coli* (0.5%). *Gram-negative bacteria* were dominated by *Pseudomonas aeruginosa* (102 strains,6.7%) and *Acinetobacter baumannii* (51 strains,3.3%). The drug resistance rate of *Pseudomonas aeruginosa* was lower than 35%, while the drug resistance rate of *Acinetobacter baumannii* to ceftazidime was more than 80%. Coagulase-negative staphylococcus (831 strains,52.5%) and *Staphylococcus aureus* (348 strains,22.0%) were isolated from gram-positive bacteria. The detection rate of coagulase-negative staphylococcus was greatly increased. Methicillin-resistant *Staphylococcus aureus* (*MRSA*) and methicillin-resistant Coagulase-negative Staphylococcus (*MRCNS*) of were 33.3% and 62.1%, respectively. **Conclusion:** Our hospital should pay more attention to the detection of bacterial drug resistance so as to provide valuable drug sensitivity results for clinical practice.

1. Introduction

In recent years, with the extensive use of antibacterial drugs, the drug resistance of pathogenic bacteria is becoming more and more serious. Under the background that the detection rate of drug-resistant bacteria is increasing year by year and the development of new antibacterial drugs is not ideal, the problem of bacterial drug resistance has become the focus of global anti-infection treatment. It has become a top priority for hospitals to strengthen the dynamic monitoring of drug-resistant bacteria, master their changes and provide reference for rational clinical drug use[1].In order to provide guidance for clinical drug use, retrospective analysis of pathogenic bacteria in recent three years was conducted in our hospital.

2. Materials and Methods

2.1 Material

2.1.1 Source

3586 strains of pathogenic bacteria were selected from clinical samples collected from the Second Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine from January 1, 2018 to December 31, 2020, and repeated strains in the same part of the same patient were eliminated.

2.1.2 Instruments and reagents

Xinke biological bacteria identification/drug sensitivity analysis system, Yixin Bochuang time of flight mass spectrometer, antibacterial drug sensitivity test paper is the products of Wenzhou Kangtai Biological Technology Co., LTD.

2.2 Methods

2.2.1 Bacterial isolation and identification and drug sensitivity test

Bacteria identification and drug sensitivity were detected by Xinke Biological bacteria identification/drug sensitivity analysis system or Yixin Bochuang time of flight mass spectrometer. The sensitivity results were interpreted in accordance with the 2020 guidelines of the American Committee for Clinical Laboratory Standardization. The quality control strains were *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 and *Staphylococcus aureus* ATCC29213.

2.2.2 Statistical analysis

The drug sensitivity test results and drug resistance were statistically analyzed by WH0NET5.6 software.

3. Results

3.1 Distribution of pathogenic bacteria

A total of 3586 strains were isolated from January 1, 2018 to December 31, 2020, including 1582 gram-positive strains (44.1%). 1542 strains of *Gram-negative bacteria* accounted for 43.0%; 462 strains of F*ungi*, accounting for 12.9%, as shown in Table 1; Sputum samples ranked first, accounting for 21.0%, followed by puncture fluid and urine, as shown in Table 2. Department of breast, department of respiratory, department of gynecology and ICU were in the top four of pathogen detection, with rates of 17.2%, 11.0%, 10.3% and 6.6%, respectively.

Table 1: Species and composition ratio of Pathogenic bacteria (n=3586) [Strains (%)]

Pathogenic bacteria	2018 year	2019 year	2020 year	Total
Gram-negative Bacterium	434(53.1)	532(37.0)	576(43.2)	1542(43.0)
Escherichia coli	141(17.3)	168(11.7)	238((17.9)	547(15.3)
Klebsiella pneumoniae	60(7.3)	88(6.1)	108(8.1)	256(7.1)
Haemophilus influenzae	71(8.7)	69(4.8)	29(2.2)	169(4.7)
Pseudomonas aeruginosa	23(2.8)	36(2.5)	43(3.2)	102(2.8)
Klebsiella oxytoca	19(2.3)	36(2.5)	36(2.7)	91(2.5)
Stenotrophomonas maltophilia	17(2.1)	17(1.2)	17(1.3)	51(1.4)
Baumanii	17(2.1)	23(1.6)	11(0.8)	51(1.4)
Enterobacter cloacae	12(1.5)	15(1.0)	20(1.5)	47(1.3)
Other	74(9.1)	80(5.6)	74(5.6)	228(6.4)
Gram-positive Bacteria	253(31.0)	723(50.3)	606(45.5)	1582(44.1)
Coagulase negative staphylococcus	71(8.7)	427(32.8)	333(25.0)	831(23.2)
Staphylococcus aureus	72(8.8)	161(11.2)	115(8.6)	348(9.7)
Enterococcus faecium	27(3.3)	52(3.6)	58(4.4)	137(3.8)
Other	83(10.2)	83(5.8)	100(7.5)	266(7.4)
Fungus	130(15.9)	182(12.7)	150(11.3)	462(12.9)
Candida albicans	57(7.0)	70(4.9)	79(5.9)	206(5.7)
Aspergillus niger	26(3.2)	26(1.8)	11(0.8)	63(1.8)
Other	47(5.8)	86(6.0)	60(4.5)	193(5.4)
Total	817(100)	1437(100)	1332(100)	3586(100)

Table 2: Source distribution of pathogenic bacteria [Strains (%)]

Source	2018 year	2019 year	2020 year	Total
sputum	218(26.7)	281(19.6)	254(19.1)	753(21.0)
Puncture fluid (Mammary Disease department: 358)	70((8.6)	392(27.3	99(7.4)	561(15.6)
urine	139(17.0)	195(13.6)	210(15.8)	544(15.2)
blood	106(13.0)	111(7.7)	133(10.0)	350(9.8)
nipple discharge	0(0)	17(1.2)	219(16.4)	236(6.6)
vaginal secretion	45(5.5)	92(6.4)	96(7.2)	233(6.5)
throat swab	78(9.5)	46(3.2)	24(1.8)	148(4.1)
External auditory canal secretion	41(5.0)	62(4.3)	22(1.7)	125(3.5)
Wound exudate	15(1.8)	39(2.7)	65(4.9)	119(3.3)
irrigating solution	17(2.1)	28(1.9)	30(2.3)	75(2.1)
other	88(10.8)	174(12.1)	180(13.5)	442(12.3)
Total	817(100)	1437(100)	1332(100)	3586(100)

3.1.1 Analysis of drug resistance of Gram-negative bacteria

Enterobacteriaceae

Enterobacteriaceae bacteria are mainly *Escherichia coli* and *Klebsiella pneumoniae*. The resistance rate of *Escherichia coli* to ampicillin quinolone sulfonamides and the 1st, 2nd, 3rd and 4th generation cephalosporins was mostly > 50%, and showed a slight downward trend in 3 years, most of the imipenem aminoglycoside enzyme inhibitor drugs and furantoin (urine specimens). Among them, 7 strains of pathogenic EScherichia coli were detected in feces. The drug resistance rates of *Klebsiella pneumoniae* and *Klebsiella oxytoca* were mostly lower than 50% in 3 years, but the drug resistance rates of *Klebsiella pneumoniae* to most cephalosporins and sulfa drugs showed an upward trend, while the drug resistance rates of *Klebsiella oxytoca* showed a downward trend basically, as shown in Table 3 for details. The isolation rates of *Extended Spectrum β Lactamase* (*ESBLs*) producing *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were 60.0%, 29.3% and 40.7%, respectively. During 3 years, the detection rate of *ESBLs* in *Escherichia coli* decreased from 63.8% to 56.7%, and that in *Klebsiella pneumoniae* increased from 25.4% to 33.3%. The detection rates of carbapenem-resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were

0.5%, 2.3% and 5.5% respectively. In addition, 47 strains of *Enterobacter cloacae* were detected, mostly from ICU, including 25 strains producing Ampc enzyme, 4 strains resistant to carbapenemase, and 1 strain multidrug-resistant. The detection rate of enterobacteriaceae cloacae was low, but the proportion of drug-resistant bacteria was 63.8%.

Table 3: Resistance rate of Enterobacter to antibiotics (%)

Antibacterial agents	*Escherichia coli* (n=547)			*Klebsiella pneumoniae* (n=256)			*Klebsiella oxytoca* (n=91)		
	2018	2019	2020	2018	2019	2020	2018	2019	2020
cefoperazone	63.8	61.3	56.7	24.6	28.4	33.3	47.4	52.8	25.0
cefoperazone and Sulbactam	2.9	5.4	5.5	6.9	6.8	0.9	10.5	13.9	5.6
cefuroxime	69.8	60.6	55.9	36.2	32.1	38.0	47.4	61.1	25.0
piperacillin/tazobactam	4.3	5.4	6.3	6.7	9.1	3.7	15.8	13.9	5.6
ceftazidime	61.7	58.7	54.6	25.4	26.1	31.5	36.8	50.0	22.2
cefepime	46.1	40.1	54.2	15.0	18.2	31.5	31.6	33.3	11.1
imipenem	0	0.6	0.8	1.7	4.6	0.9	5.3	11.1	0
amikacin	3.6	0.6	3.5	1.7	7.8	4.7	5.3	16.7	2.8
levofloxacin	58.9	43.3	42.0	11.7	17.2	12.0	26.3	47.2	16.7
cefoxitin	12.3	6.0	6.3	11.9	5.7	1.9	15.8	13.9	5.6
cefotaxime	61.7	58.9	55.5	24.1	27.3	33.3	36.8	52.8	27.8
gentamicin	45.3	46.9	38.4	15.5	25.0	17.9	47.4	30.6	16.7
SXT	60.3	59.0	55.7	21.7	24.4	28.4	16.8	17.2	25.0
furantoin (urine specimen)	6.7	1.5	3.0	12.5	15.4	14.3	16.8	17.2	11.4
ampicillin	90.9	100	91.7	——	——	——	——	——	——
cefazolin	86.6	84.2	62.2	33.3	31.8	36.1	86.2	72.2	50.0

Note: indicates that the bacterium is naturally resistant to the drug, and the drug resistance rate does not include intermediaries.

Non-fermentative bacteria

Non-fermentative bacteria were mainly *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The drug resistance rates of *Pseudomonas aeruginosa* were all lower than 35%, but most of them showed an upward trend. The drug resistance rate of *Acinetobacter baumannii* to ceftazidime was more than 80%, and the drug resistance rate of polymyxin minocycline was less than 30%, as shown in Table 4 and 5. In addition, rare bacteria were detected :1 strain of *Elisabella meningosepticum* from neonatal cerebrospinal fluid and 1 strain of *Elisabella meningosepticum* from surgical wound secretion.

Table 4: Resistance rate of Pseudomonas aeruginosa to antibiotics (%)

Antibacterial agents	*Pseudomonas aeruginosa* (n=102)		
	2018 year	2019 year	2020 year
piperacillin	26.3	19.4	32.6
piperacillin/tazobactam	10.0	5.6	18.6
ceftazidime	21.7	25.0	25.6
aztreonam	21.7	19.4	20.9
cefoperazone and sulbactam	0	5.6	14.0
cefepime	0	5.6	7.0
imipenem	0	22.2	18.6
meropenem	0	18.4	15.2
gentamicin	16.7	16.7	16.7
amikacin	4.4.	2.9	7.0
levofloxacin	8.7	11.4	7.0
polymyxin	0	0	4.7

Table 5: Resistance rate of Acinetobacter baumannii to antibiotics (%)

Antibacterial agents	*Acinetobacter baumannii* (n=51)		
	2018 year	2019 year	2020 year
piperacillin/tazobactam	47.1	78.3	55.6
ceftazidime	82.4	91.3	81.8
cefoperazone and sulbactam	29.4	34.8	27.3
cefepime	41.2	78.3	45.5
imipenem	47.1	74.0	36.4
meropenem	49.5	69.3	31.4
minocycline	14.3	27.3	10.0
amikacin	29.4	10.0	9.1
levofloxacin	29.4	77.3	40.0
polymyxin	0	0	0

Haemophilus influenzae

The isolation rate of *Haemophilus influenzae* was the third in *Gram-negative bacteria*, next only to *Escherichia coli* and *Klebsiella pneumoniae*. 99.4% were isolated from upper respiratory tract specimens.During 3 years, the resistance rates of *Haemophilus influenzae* to cefuroxime ceftriaxone levofloxacin and azithromycin were<30%, more to sulfonamides >50%.

3.1.2 Analysis of drug resistance of common Gram-positive Bacterium

348 strains of *Staphylococcus aureus* and 831 strains of coagulase-negative Staphylococcus aureus were isolated, and methicillin-resistant *Staphylococcus aureus* was included in *Staphylococcus aureus* (*MRSA*) and coagulase-negative staphylococcus (*MRCNS*) were 33.3% and 62.1%. *MRSA Methicillin sensitive Staphylococcus aureus*, respectively. *MSSA*, *MRCNS* and Methicillin sensitive coagulase-negative *Staphylococcus.* The drug resistance rates of *MRSA* to antibiotics were all higher than MSSA (except teicoranin). The drug resistance rates of *MRCNS* to antibiotics were all higher than *MSCNS* (see Table 6 for details). In addition, 7 strains of linezolid resistant *Staphylococcus* and 2 strains of teicoranin resistant *Staphylococcus* were detected.

Table 6: Resistance rate of Staphylococcus to antibiotics (%)

Antibacterial agents	*Staphylococcus aureus* (n=348)		*Coagulase negative staphylococcus* (n=831)	
	MRSA	*MSSA*	*MRCNS*	*MSCNS*
penicillin	95.7	90.0	97.8	86.4
erythromycin	88.7	68.0	92.1	85.1
clindamycin	79.1	51.5	64.6	48.3
linezolid	1.7	0.4	0.6	0.3
levofloxacin	23.5	3.9	32.8	10.9
rifampicin	8.7	0.9	5.9	2.0
cotrimoxazole	13.0	7.5	32.2	17.2
amikacin	7.8	2.6	4.9	1.0
teicoplanin	0	0.4	0.2	0
tetracycline	43.5	7.4	35.6	25.6
gentamicin	22.6	13.0	34.3	19.2

Enterococcus

A total of 137 *Enterococcus faecium* strains (8.7%) and 25 *Enterococcus faecium* strains (1.6%) were detected in *Gram-positive bacteria*. The drug resistance rates of *Enterococcus faecium* to

penicillin, levofloxacin furantoin high concentration gentamicin and rifampicin were > 50%, except rifampicin, the drug resistance rate of *Enterococcus faecalis* to other antibacterial drugs was lower than 40%. No *Enterococcus faecalis* resistant to vancomycin teicoranin was detected, no *Enterococcus faecalis* resistant to vancomycin and teicoplanin was detected. One strain of *Enterococcus faecium* resistant to teicoplanin, three strains of *Enterococcus faecium* resistant to linezolid and one strain of *Enterococcus faecium* resistant to linezolid were detected.

A total of 36 strains of *Gram-positive bacteria* were detected, including 20 strains (55.6%) from adults over 60 years old and 10 strains (23.8%) from children under 10 years old. Most of them were from sputum and other upper respiratory tract samples (29 strains,80.6%), followed by blood samples (5 strains,13.9%) and puncture fluid (2 strains,5.6%, both from mammary gland). In 3 years, the resistance rate of *Streptococcus pneumoniae* to penicillin and erythromycin was> 50%. Penicillin resistant *Streptococcus pneumoniae* (*PRSR*), erythromycin-resistant *Streptococcus Pneumoniae* (*ERSP*), was sensitive to levofloxacin, vancomycin, cefuroxime, ceftriaxone and meropenem.

4. Discussion

The detection results showed that the detection rate of *Gram-positive bacteria* in our hospital was high, followed by *Gram-negative bacteria* and *Fungi. Escherichia coli, Staphylococcus aureus, klebsiella pneumoniae, Staphylococcus epidermidis* and *Haemophilus* influenzae in the top five. Sputum was the main specimen, followed by puncture fluid. The respiratory department of the Department of Gynecology of the Department of Breast Diseases is located in the top three of the department distribution, and the detection results are different from the existing research results in China[2,3], which may be caused by the differences between regions and hospitals. Among *Enterobacteriaceae*, *Escherichia coli* is mainly derived from urine specimens, while *Klebsiella pneumoniae* is mainly derived from sputum specimens. Among intestinal and non-fermentation pathogens, β-lactam antibiotics are widely used as therapeutic drugs. With the long-term use of antibacterial drugs, the incidence of *ESBLs* strains in pathogens is increasing[4-6], among which *Escherichia coli.* The detection rates of *ESBLs* production by *Klebsiella pneumoniae* and *Klebsiella oxytoca* were 60.0%, 29.3% and 40.7%, respectively During the 3 years, the detection rate of *ESBLs* in *Escherichia coli* decreased year by year, and that in *Klebsiella pneumoniae* increased year by year, which may be related to the elderly patients with underlying diseases and low immunity. The detection rates of *ESBL*-producing acid-producing *Klebsiella* and carbapenem-resistant acid-producing *Klebsiella* were higher than those of *Klebsiella pneumoniae*, suggesting that hospitals should pay attention to distinguish acid-producing *Klebsiella pneumoniae* from *ESBL*-producing *klebsiella* and continue to summarize their drug resistance and analyze their characteristics, providing reference for clinical drug use. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were mostly derived from sputum specimens and were often isolated from pneumonia patients (especially ventilator-associated pneumonia). The drug resistance rates of *Pseudomonas aeruginosa* to antibiotics were >35%, and most of them show an upward trend, which is different from existing domestic research results [7]. Penicillium carbon alkene resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were 15.7% and 56.9% respectively, the ratio of multiple drug-resistant bacteria, is common in clinical practice in the patient's body to produce biofilm, biological membrane protective effect to improve the drug resistance of bacteria to antimicrobial agents and evade the immune system to attack[8, 9], so how to suppress the formation of biofilm is the present clinical treatment studies have found that Traditional Chinese medicine can inhibit the formation of biofilm of film-producing strains[10-12], improve the permeability of antibacterial drugs, and effectively play their killing effect. The drug resistance rate of penicillin G in staphylococcus was >85%, so it can not be used as a common drug in clinic. Coagulase-negative Staphylococcus and *Staphylococcus aureu*s detection rate respectively

in gram-positive bacterium of the first and the second specimen, mainly for mammary gland diseases for puncture fluid and nipple discharge, *MASA* detection rate was 33.3%, and the national average basic same[7].Coagulase-negative Staphylococcus detection rate increased significantly, on the one hand is caused by Coagulase-negative staphylococcus is human body normal flora of the skin, may be due to improper collection, specimen exposed to the skin, causing false positives, on the other hand, due to the breast surgery and ductal invasive operations, lien barrier damage to human body, cause traumatic infections[13].

5. Conclusion

In summary, hospitals should attach great importance to the infection of multidrug-resistant bacteria to avoid cross-infection and outbreak of epidemics in hospitals. Standardize the collection of sterile body fluids to reduce the false positive of test specimens. Clinical laboratory timely collate and feedback the results of drug resistance of pathogenic bacteria to guide clinical rational drug use. Clinicians should not only refer to the results of drug sensitivity, but also pay attention to the patient's signs and history to avoid abuse of antibacterial drugs.

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