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Research on pathogenic bacteria and antibiotic resistance of Enterobacteriaceae in hospitalized elderly patients.

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Abstract

Objective: To observe the pathogens types of Enterobacteriaceae infection in hospitalized elderly patients, and analyse its drug resistance of germ and provide laboratory evidence for clinical prevention and treatment in the future.

Methods: The clinical data of hospitalized elderly patients (≥ 60 y) from July, 2009 to June, 2017 were retrospectively analysed, identification of the strain was conducted after using conventional methods for separation of Enterobacteriaceae, and the disk diffusion method (K-B) was adopted for detecting the drug resistance of bacteria to various antimicrobial agents.

Results: In this study, 3541 cases were confirmed with the occurrence of hospital infection from Enterobacteriaceae, and 2338 strains of Enterobacteriaceae were isolated, of which 771 strains of *Escherichia coli* (32.98%), 682 strains of *Klebsiella* (29.17%) with the highest relevance ratio. The drug resistance rates of *Escherichia coli* to sulfamethoxazole were the highest (98.57%) and that of *Klebsiella pneumoniae* reached 82.99%. They were followed by ciprofloxacin and levofloxacin. The drug resistance rates of *Escherichia coli* to ciprofloxacin and levofloxacin were higher than *Klebsiella pneumoniae* ($p<0.05$). In every sample, the drug resistance rates of sulfamethoxazole, ciprofloxacin and levofloxacin were relatively high. The drug resistance of cefotaxime and piperacillin in the drainage liquid samples were higher than other, but that of sulfamethoxazole was lower than the urine samples ($p<0.05$). The drug resistance of levofloxacin, imipenem, meropenem and sulfamethoxazole in the sputum sample were lower than urine samples ($p<0.05$).

Conclusion: The Enterobacteriaceae bacteria produce the drug resistance to many antibiotics, which affects the treatment effect of hospitalized elderly patients. The antibacterial agents should be properly used and the distribution condition of pathogenic bacteria and drug resistance of patients should be closely monitored.

Keywords: Elderly, Enterobacteriaceae, Bacterial infections, Pathogens, Drug resistance.

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Introduction

At present, with the infection degree of hospital pathogens continuing to expand, the effective pathogen prevention and control has become the main task for clinicians. Due to clinicians' and patients' irrational use of broad-spectrum antibiotics, the drug resistance of most pathogenic bacteria are increasing [1]. So the control of intestinal bacteria is very important. Because of physical function constant decline and resistance reduction of elderly patients who are suffering from a variety of illness and are more susceptible to infection and do not have a good respond to treatment with long time of hospitalization, so the control of bacterial infections in hospitalized elderly patients is more important [2-4]. Therefore, the pathogenic bacteria and drug resistance of hospitalized elderly patients with Enterobacteriaceae infection in our hospital were studied in this research and the drug resistance rate was reduced as possible for clinical rational drug use, in order to provide the basis for improving the clinical cure rate and survival rate in the future. The current study report is shown as follows.

Data and Methods

General information

The hospitalized elderly patients (≥ 60 y old in our hospital from July, 2009 to June, 2017 were included in the study for retrospective analysis according to 'the diagnostic criteria for hospital infection' formulated by the National Health Planning Commission [5] combined with medical history, symptoms, signs and imaging examination report and medical examination reports. In these cases, 541 were confirmed for occurrence of *Escherichia coli* hospital bacterial infection, whose inspection samples came from infection samples of elderly hospitalized patients, of which there were 876 copies of urine, 813 of sputum samples, 642 of drainage samples, 589 of secretions samples, 304 cases of pus samples, 317 cases of others. After

excluding isolated strains from the same parts of patients where he agreed (agreed strains collected in 7 d, which were not repeatedly included in the statistics), and through VITEK2 compact automatic microbial analysis system (provided by the BioMerieux company in Lyons, France), 2338 strains of Enterobacteriaceae were isolated and collected. The study and patients or their relatives signed informed consent form.

Test supplies

In this study, the antibacterial drug disk were purchased from Thermo Fisher biochemical products (Beijing) company, MH medium was purchased from the British company OXOID, as well as ceftazidime clavulanic acid, cefotaxime clavulanic acid and nitrocefin disk from OXOID goods, which were 30 μg/piece. Disposable medical supplies were purchased from Beijing Hua Rui Bo Yang Technology Co. Ltd. All products were qualified to enter the indoor quality control before becoming clinical specimens.

Bacterial culture and isolation

In this study, the isolated 2338 strains were cultured by the microbiological inspection technology in the national clinical laboratory operation procedures. The collected strains were inoculated in the culture dish and cultured for 18-24 h in 35°C for their recovery. After the single bacterial colony was obtained, all the *Escherichia coli* were further evaluated using automatic VITEK2 microorganism identification drug sensitivity instrument according to gram stain, bacteria form. All the specimens were cultured by the same group of personnel, and cultured samples were transported and identified by certain person.

Strain identification and drug susceptibility test

In this study, the cultured strains were identified by VITEK2 compact automatic microbial analysis system, and relative inspection standard and operation specification M02-A11: 'standard for antimicrobial susceptibility testing operating procedures' (disk method) were used for preliminary screening and phenotypic confirmatory test. The bacteria liquid of 0.5 Maxwell was formulated using sterile normal saline and uniformly coated on the M-C agarose surface with 4% NaCl. The drug sensitivity paper pieces of amoxicillin, amikacin, ceftazidime, cefotaxime, cefepime, ciprofloxacin, levofloxacin, imipenem, meropenem, ertapenem, sulfamethoxazole, gentamicin, sulbactam, piperacillin were put on the M-H agar surface and incubated for 24 h in the constant temperature water incubator in 35°C using paper separator. The bacterial inhibition diameters were respectively detected.

Quality control strains

In this study, *Escherichia coli* (ATCC25922) and *Klebsiella pneumoniae* (ATCC 700603) were selected as the quality control strains, all of which were derived from the clinical laboratory center of the Ministry of health. The standard strains (ATCC25922 and ATCC 700603) using antibacterial drug paper every week were detected and their results were within the interpretation range specified by CLSI.

Statistical data analysis

All the data were input into Excel spread sheet for statistical analysis by using SPSS18.0 statistical software for data processing. The χ^2 test was used for count data, and $P<0.05$ meant the difference was statistically significant. Drug resistance test results were analysed by WHONET 5.6 software.

Result

Bacterial distribution

In this study, 3541 cases were confirmed with the occurrence of hospital infection from Enterobacteriaceae, and 2338 strains of Enterobacteriaceae were isolated, of which 771 strains of *Escherichia coli* (32.98%) , 682 strains of *Klebsiella pneumoniae* (29.17%) with the highest detection rate, 465 strains of *Enterobacter* spp. (19.89%), 249 strains of *Klebsiella oxytoca* (10.65%), and other 171 strains (7.31%) were detected. The results are shown in Table 1.

Table 1. *Distribution of pathogenic bacteria.*

Pathogenic bacteria	Number (stain)	Constituent ratio (%)
Escherichia coli	771	32.98
Klebsiella pneumoniae	682	29.17
Enterobacter	465	19.89
Klebsiella oxytoca	49	10.65
Others	171	7.31
Total	2338	100%

Specimen distribution

In this study, samples infected by Enterobacteriaceae bacterial included 587 urine samples (25.11%), 423 sputum samples (18.09%), 396 drainage samples (16.94%), 369 secretions samples (15.78%), 295 pus samples (12.62%) and 268 other samples (11.46%) (Table 2).

Table 2. *The distribution and composition of bacterial specimens (%).*

Specimen	Number (stain)	Constituent ratio (%)
Urine	587	25.11
Sputum sample	23	18.09
Drainage sample	369	16.94
Secretion sample	369	15.78
Pus sample	295	12.62
Others	268	11.46

Detection rate of drug-resistant bacteria

In this study, 771 strains of *Escherichia coli* were detected in 2338 strains of Enterobacteriaceae, and 682 strains of *Klebsiella pneumoniae* were detected. Drug resistance analysis on the quality control index of *Escherichia coli* and *Klebsiella pneumoniae* was conducted and the results showed that the drug resistance rate of *Escherichia coli* to sulfamethoxazole was the highest upto 98.57% and that of *Klebsiella pneumoniae* was 82.99%, followed by ciprofloxacin and levofloxacin with respective 85.08% and 80.42% resistant rates of *Escherichia coli*, which were higher than *Klebsiella pneumoniae* (66.86%, 50.0%). There was statistic difference ($p<0.05$). Then gentamicin, cefotaxime, cefepime, ceftazidime amoxicillin appeared. And the resistance rates of other drugs with higher sensitivity such as Amikacin, piperacillin and sulbactam were all <25%. The detection results of *Klebsiella pneumoniae* were similar to that of *Escherichia coli*. But drug resistance rates of cefotaxime, cefepime, ciprofloxacin, levofloxacin were lower than *Escherichia coli* and there was statistic difference ($p<0.05$) (Table 3).

Analysis of drug resistance rate in different samples

In the clinical study, the resistance rates of *Escherichia coli* to common drugs in 3541 samples were analysed, and the results showed that, in urine samples, the resistance rates of sulfamethoxazole, ciprofloxacin and levofloxacin were higher, the resistance rates of amikacin, piperacillin and sulbactam were relatively lower. The trends of drug resistance in sputum, drainage, secretion, pus and other samples were similar. Additionally, the drug resistance rates of cefotaxime and piperacillin in the drainage liquid samples were higher than other samples. But that of Sulfamethoxazole was lower than urine samples and there was statistic difference ($p<0.05$). The drug resistance rates of levofloxacin, imipenem, meropenem and Sulfamethoxazole were lower than urine samples and there was statistic difference ($p<0.05$) (Table 4).

Table 3. *Drug resistance (%) of major intestinal bacteria.*

Antimicrobial agents	*Escherichia coli* (n=771)		*Klebsiella pneumoniae* (n=682)	
	Number	Resistance rate	Number	Resistance rate
Amoxicillin	423	54.86	319	6.77
Amikacin	122	15.8	88	12.9
Ceftazidime	416	53.96	234	34. 31a
Cefotaxime	518	67.19	357	52. 35a
Cefepime	78	62.0	342	50.15
Ciprofloxacin	656	85.08	456	66.86a
Levofloxacin	620	80.42	341	50.0a
Imipenem	0	0.0	0	0.0
Meropenem	0	0.0	0	0.0
Ertapenem	0	0.0	0	0.0
Sulfamethoxazole	760	98.57	566	82.99
Gentamicin	539	69.91	338	49.56a
Sulbactam	177	22.96	146	21.41
Piperacillin	123	15.95	129	18.91

Note: a was compared with *Escherichia coli*, $p<0.05$;

Table 4. *Analysis of drug resistance rate of Escherichia coli in different samples (n (%)).*

Antimicrobial agents	Urine (n=587)	Sputum sample (n=423)	Drainage sample (n=396)	Secretion sample (n=369)	Pus sample (n=295)	Others (n=268)
Amoxicillin	66 (11.24)	28 (6.62)	19 (4.80)	58 (15.72)	33 (11.19)	32 (11.94)
Amikacin	37 (6.30)	27 (6.38)	29 (7.32)	47 (12.74)	9 (3.05)	21 (7.84)
Ceftazidime	18 (3.07)	9 (2.13)^{e}	0 (0.00%)^{e}	79 (21.41)^{b}	0 (0.00%)^{e}	4 (1.49)^{e}
Cefotaxime	46 (7.84)	63 (14.89)	116 (29.29)	0 (0.00%)^{cd}	26 (8.81)^{d}	5 (1.87)^{d}
Cefepime	28 (4.77)	26 (6.15)	18 (4.55)	1 (2.71)	8 (2.71)	13 (4.85)
Ciprofloxacin	39 (6.64)	26 (6.15)	0 (0.00%)	0 (0.00%)	24 (8.14)	0 (0.00%)
Levofloxacin	85 (14.48)	8 (1.89)^{b}	30 (7.56)	0 (0.00%)^{bg}	32 (10.85)	33 (12.31)
Imipenem	9 (1.53)	46 (10.87)	8 (2.02)	19 (5.15)	0 (0.00%)^{c}	0 (0.00%)^{c}
Meropenem	17 (2.090)	75 (17.73)	3 (7.83)	19 (5.15)	26 (8.81)	26 (9.70)
Ertapenem	18 (3.07)	21 (4.96)	9 (2.27)	28 (7.59)	32 (10.85)	41 (15.30)^{be}
Sulfamethoxazole	117 (19.93)	29 (6.86)^{b}	17 (4.29)^{b}	7 (1.90)^{b}	17 (5.76)^{b}	8 (2.99)^{b}
Gentamicin	9 (1.53)	18 (4.26)	16 (4.04)	16 (4.34)	6 (2.03)	0 (0.00%)

Sulbactam	84 (14.31)	47 (11.11)	38 (9.60)	18 (4.88)	29 (9.83)	30 (11.19)
Piperacillin	8 (1.36)	7 (2.65)	58 (14.65)[bc]	39 (10.57)	44 (14.92)[bc]	9 (3.36)

Note: [b]was compared with urine samples, p<0.05; [c]was compared with drainage liquid samples, p<0.05; [e]was compared with secretion samples, p<0.05; [g]was compared with other samples, p<0.05.

Discussion

Currently, because of irregular and unreasonable use of clinical antibacterial agents, the drug resistance of bacterial is increasing, which seriously affects the treatment effect of drug [6]. In the study, the detection rate of enterobacteriaceae bacteria in elderly patients in our hospital was 66.03% accounting for a large proportion. Especially, *Escherichia coli* and *Klebsiella pneumoniae* were main detected stains with 32.98% and 29.17% of detection rate, which should be regarded as mainly focused bacterial infected and monitored by the hospital. Antibacterial agents play a very import role in use number, amount, variety and other aspects of our clinic and ward. They save life and health of many patients meanwhile promoting the production of "superbacteria", which is mainly caused by abuse, misuse, joint use and irregular and unreasonable use of antibiotic [7]. To this day, the drug resistance and study bottleneck of antibacterial agents have become one difficulty urgently needing to be solved, otherwise, which may form an awkward situation of "no medicine".

In this study, the drug resistance rate of gram-negative bacterium *Escherichia coli* to sulfamethoxazole was the highest up to 82.99%, followed by ciprofloxacin and levofloxacin. The drug resistance rates of *Escherichia coli* to Ciprofloxacin and levofloxacin were higher than *Klebsiella pneumoniae* and there was statistic difference ($p<0.05$). Then gentamicin, cefotaxime, cefepime, amoxicillin, ceftazidime were followed. The drug resistance rates of other high sensitivity drugs (such as amikacin, piperacillin and sulbactam) were lower than 25%. The detection result of *Klebsiella pneumoniae* was similar to *Escherichia coli*. But the drug resistance rates to ceftazidime, cefotaxime, ciprofloxacin and levofloxacin were lower than *Escherichia coli* and there was statistic difference ($p<0.05$). We can see that we can select more reasonable treatment scheme by timely and correctly understanding pathogenic bacteria of elderly patients and drug resistance monitoring. The hyper spectrum β-lactamase (ESBLs) is one of important reasons that why gram-negative bacillus produces drug resistance to penicillin and cephalosporin [8,9]. Certain study shows that currently, the detection rate of the enzyme is increasing year by year [10]. Once the bacteria produced the antibacterial ESBLs enzyme, they would almost be resistant to penicillins, cephalosporins, etc., and only sensitive to carbapenem antibiotics. Therefore, once the resistance to the drug appears, it will bring a new crisis to clinical treatment [11]. It is worth noting that the United States has found the bacteria resistant to antibiotic carbapenem in 2001, and reports appeared in China in 2009 which showed antibacterial drugs resistant to carbapenems had gradually lost its advantage [12-14]. But it is worth mentioning that, in this study, we have not detected bacteria's drug tolerance to carbapenem antibiotics such as imipenem, meropenem and ertapenem, the results of which was consistent with Xu [15] and other study results, so that this kind of drug can also be used for clinical promotion and replacement. The clinical study found that Enterobacteriaceae produced multiple drug resistance and cross resistance to quinolones and aminoglycoside, which may be related to irregular use of antibiotic, so that the corresponding laws should be formulated to curb this situation [16].

In summary, in the clinical research of antibacterial drugs, detection of bacterial resistance is an important way to observe the clinical rational use of drugs, the laboratory should establish a routine method to make timely detection of them, and make rational clinical guided medication by using the results from this study as well as control the spread of drug resistant strains. Thus, in clinical treatment, drug resistance of the pathogen plays a particularly important role in the course of treatment, and with the increase of antimicrobial resistance of common bacteria, the rational use of antimicrobial drugs is an important way to control bacterial infection and outbreak.

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