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Evaluation of Resistance by Clinically Pathogenic Bacteria to Antimicrobials and Common Disinfectants in Beijing, China

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Background: Antibiotic resistance of pathogenic bacteria is well recognized among clinicians; however, studies that directly evaluate the bacterial resistance to commonly used disinfectants in clinical settings are lacking. Currently available reports focus on the resistance of single strains to single disinfectants and do not adequately examine the degree of resistance and cross-resistance to antimicrobials in the large-scale clinical use of disinfectants.

Methods: We investigated the resistance capacity to 11 antibiotics and 7 chemical disinfectants by bacterial strains collected from body fluids of patients in 10 hospitals in Beijing, China over a 1-year period. Bacterial resistance to disinfectants was tested using minimum inhibitory concentration and minimum bactericidal concentration using agar dilution methods based on commercially available reference strains.

Results: A total of 1,104 pathogenic strains were identified, of which 23% were Gram-positive bacteria, 74% were Gram-negative bacteria, and 3% were fungi. Overall, resistance to antibiotics for the most common strains was significantly higher than their resistance to disinfectants. The least effective antibiotics and disinfectants were aztreonam and glutaral, respectively, exhibiting the highest overall resistance rates; while amikacin and alcohol had the lowest resistance rates. Consistently, *Acinetobacter baumannii* exhibited the most resistance, while *Escherichia coli* had the least resistance for both antibiotics and disinfectants.

Conclusions: Based on the pathogen spectrum for bacterial infective pathogens evaluated in this study, as well as the status quo of their resistance to antimicrobial agents and common clinical disinfectants, it is essential for healthcare professionals to pay attention not only to the standardized use of antimicrobial agents but also to the rational application of disinfectants. (J Nippon Med Sch 2018; 85: 302–308)

Key words: antimicrobials, clinical surveillance, drug resistance, hospital isolates, pathogenic bacteria

Introduction

The inappropriate use of disinfectants results in bacterial resistance to disinfectants[1], and both intrinsic and acquired bacterial resistance mechanisms have been identified that contribute to this process[2]. Contaminated hospital surfaces and equipment are common pathogen reservoirs, especially for drug-resistant strains, which can increase hospital-acquired infections (HAIs). Consequently, HAIs further prolong patient hospitalization, increase morbidity and mortality, and have an undue impact on

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Table 1 Disinfectants and corresponding neutralizing solutions

Disinfectant	Manufacturer	Neutralizing solution
2% glutaral	Chinese Shanghai Likang Disinfection Technology Co., Ltd.	500 mL PBS+2.5 g glycine+2.5 g lecithin+2.5 g Tween-80
2% iodine tincture	Chinese Shandong lierkang Disinfection Technology Co., Ltd.	1.0% sodium thiosulfate
1% iodophor	Chinese Shandong lierkang Disinfection Technology Co., Ltd.	1,000 mL PBS+sodium thiosulfate 10 g
75% alcohol	Chinese Fujian Putian Pharmaceutical Alcohol Co., Ltd.	500 mL PBS+1.5 g Tween-80
6% sodium hypochlorite	Chinese Henan Hualong Pharmaceutical Co., Ltd.	1.0% sodium thiosulfate
0.2% benzalkonium bromide	Chinese Shandong Rui Teige Washing Disinfection Technology Co., Ltd.	5.0% Tween-80
0.1% chlorhexidine acetate	Chinese Jinzhou Jiutai Pharmaceutical Co., Ltd	5.0% Tween-80

PBS, phosphate buffered saline

healthcare costs. Therefore, there is a public health need to identify strategies that can reduce HAIs. However, studies that comprehensively assess the efficacy of clinical disinfectants in routine clinical settings are currently lacking in China. Evaluation of the efficacy of clinical disinfectants can reduce contamination burden in hospitals, which may directly lessen the incidence of HAIs. Moreover, previous studies have focused on the resistance of single pathogenic strains to single disinfectants and have not adequately evaluated the degree of resistance and/or cross-resistance to antimicrobials in the large-scale clinical use of disinfectants, as is the case in clinical settings.

In the present study, we collected clinically pathogenic bacteria from 10 hospitals in Beijing and analyzed their susceptibility to antimicrobials and chemical disinfectants. The results from this study will be useful for healthcare professionals on the optimal use of disinfectants to help reduce drug-resistant strains.

Materials and Methods

Study Centres and Ethics Statement

This study was conducted in 10 hospitals, including Ministry of Health-affiliated hospitals, city-affiliated hospitals, urban hospitals, and suburban hospitals in Beijing, China. The study was approved by the Ethics Committees and Institutional Review Boards of each hospital. Subjects provided written informed consent prior to sample collection and the study was conducted in accordance with the guidelines set forth by the Declaration of Helsinki.

Clinical Samples and Strains

Clinical pathogenic bacteria were collected from 40 clinical departments, including respiratory, critical care, emergency, infectious disease, and hematology departments, across 10 hospitals in Beijing, China between March 2014 and March 2015. The fluid samples collected were sputum, urine, blood, and pus. Only one sample was obtained from the same site for each patient during the same period.

The standard strain *Klebsiella pneumoniae* (ATCC700603) and quality control strain *Escherichia coli* (ATCC25922) were obtained from the National Institute for Control of Biological Products, and were used as antibiotic-susceptible strains. Hospital isolates and reference strains were stored at −80°C until analysis.

Disinfectants and Antibiotics

Disinfectants

The tested disinfectants were as follows: 2% glutaral, 2% iodine tincture, 1% iodophor, 75% alcohol, 6% sodium hypochlorite, 0.2% benzalkonium bromide, and 0.1% chlorhexidine acetate. Disinfectants were obtained from six companies (listed in **Table 1**) and used as-is or diluted to the above concentrations with appropriate diluents. Disinfectants were freshly prepared based on standard laboratory concentrations.

Neutralizers

Neutralizers were selected depending on the respective disinfectants[3]. See **Table 1** for details.

Antibiotics

The following antibiotics recommended by the National Committee for Clinical Laboratory Standards (NCCLS)[4] were used: Piperacillin/tazobactam, Ampicillin/sulbactam, Aztreonam, Ceftazidime, Gentamicin, Amikacin, Imipenem, Meropenem, Cefotaxime, Ciprofloxacin, and Trimethoprim/Sulfamethoxazole.

Test Methods

Testing of MIC for Antibiotics Using an Agar Dilution Method

Blood nutrient agar plates and SS media were obtained from Tianjin Jinzhang Company. Mueller-Hinton media for drug susceptibility testing was obtained from MacConkey media. The drug susceptibility testing was performed according to the protocols recommended by the Clinical and Laboratory Standards Institute (CLSI) 2015[5]. Briefly, a sterile swab was dipped in a bacterial suspension and onto Mueller-Hinton media. Antibiotic disks were then carefully placed using sterile forceps in agar plates, which were subsequently incubated at 35°C for 18 h. Drug susceptibility was classified based on the inhibition zone (in mm) as outlined in the CLSI protocols.

Detection of Resistance Using MIC and MBC

Preparation of bacterial suspension

Clinically pathogenic strains and standard strains in blood nutrient agar plates were inoculated onto the nutrient agar slant, incubated for approximately 20 h, and washed with pH 7.2 phosphate buffered saline (PBS). The bacterial solution was diluted with 1% peptone PBS to a bacterial concentration of 10^8 CFU/mL until future use.

Determination of MIC of disinfectants

Various disinfectants were diluted using doubling serial dilution into test solutions with different concentrations. For each test solution, a 2.5 mL sample with different concentrations was introduced into a test tube containing 2.5 mL nutrient broth at 2× the concentration. Then, 0.1 mL of bacterial suspension of approximately 10^8 CFU/mL was inoculated into the nutrient broth test tube containing disinfectant to serve as the test sample. The nutrient broth test tube containing no disinfectant was inoculated in the same manner to serve as the positive control sample. Two test tubes containing nutrient broth only were used as the negative control samples. The test samples, positive control, and negative control samples were incubated at 37°C for 48 h for observation.

MIC evaluation criteria

The concentration of the disinfectants corresponding to the maximum dilution strength was as follows and considered as the MIC of the sample for the test bacteria: concentration in which no bacterial growth was present in the test group; concentration in which the presence of bacterial growth in the positive control tube was indicated by turbidity; and the concentration in which no bacterial growth in the negative control tube was indicated by transparency.

Determination of MBC of disinfectants

The determination of MBC was a continuance of MIC testing. The test tubes were removed from the incubator 48 h after the above-mentioned evaluation and 0.5 mL of reaction solution was transferred to a 10 mL test tube from each test tube in the test group in which no growth of bacteria was present. Then, 4.5 mL of respective neutralizer solution was added, mixed, and incubated for 10 min to serve as the final reaction solution. The final reaction solution and the broth with 2× the concentration were added in a ratio of 1:1 to a 10 mL test tube. Neutralizer control group setting was as follows: 2.5 mL of neutralizer and 2.5 mL of broth with 2× concentration were added to a 10 mL test tube. The setting for other control groups was similar to those for the determination of MIC. These test tubes were adequately mixed and incubated for 24 h at 37°C for observation.

MBC evaluation criteria

MBC was considered as the minimum disinfectant concentration corresponding to the transparent test tube lacking turbidity in the test group if transparency was observed in the two negative control test tubes and neutralizer control test tube, while turbidity due to growth of bacteria was noted in the positive control group.

Bacterial resistance to disinfectants using MIC and MBC

The MIC and MBC for standard strains were first measured, and the measurements of other strains were compared with the standard strains in order to determine the susceptibility prior to resistance rate calculation. Resistance rate was calculated using the following formula:

$$\text{Resistance rate} = \frac{\text{Number of resistant strains}}{\text{The total number of bacteria}} \times 100\%;$$

sensitivity rate was calculated as [100 – resistance rate]%.

Quality control measures

Inoculation was performed in an ascending order of disinfectant concentration. Testing of MIC and MBC was repeated three times and the test strains and standard stains were compared for MIC and MBC. Resistance was confirmed if the MIC and MBC of the former was greater than the latter; otherwise, susceptibility was confirmed[6–9].

Statistical Analysis

The results from the drug susceptibility testing were analyzed using Whonet 5.6 (Brigham and Women's Hospital).

Results

Distribution of Isolated Strains

Samples were collected from a total of 40 patients and 1,104 strains of pathogenic bacteria were isolated from

these samples. There were 253 Gram-positive strains which accounted for 23.0% of all isolates, and comprised 130 strains of *Staphylococcus aureus* (methicillin-resistant *Staphylococcus aureus* MRSA accounting for 46.9%), 31 strains of *Staphylococcus epidermidis* (methicillin-resistant *Staphylococcus epidermidis* accounting for 47.7%), and 35 strains of *Enterococcus* (vancomycin-resistant enterococci accounting for 11.6%). In total, 817 Gram-negative strains that accounted for 74.0% of all isolates were identified, the majority including *Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Escherichia coli*. Additionally, 34 strains of fungi that accounted for 3.0% of all isolates were identified. See **Table 2** for the distribution summary of the isolated strains.

Table 2 Distribution of bacterial strains isolated from 10 hospitals

Strain	Number	%
Klebsiella pneumoniae	249	23
Pseudomonas aeruginosa	205	19
Staphylococcus aureus	130	12
Acinetobacter baumannii	67	6
Escherichia coli	60	5
Stenotrophomonas maltophilia	53	5
Proteus mirabilis	37	3
Serratia marcescens	35	3
Staphylococcus epidermidis	31	3
Staphylococcus haemolyticus	22	2
Enterobacter aerogenes	22	2
Enterobacter cloacae	19	2
Enterococcus faecalis	18	2
Enterococcus faecium	17	1
Citrobacter koseri	17	1
Others	122	11

Antibiotic Resistance of Isolated Strains

A. baumannii exhibited the least sensitivity to antibiotics in the following ascending order of susceptibility: aztreonam (22.9%), piperacillin/tazobactam (28.6%), meropenem (29.3%), imipenem (30.7%), and gentamicin (32.5%). On the other hand, *E. coli* had the most sensitivity to antibiotics in the following descending order of susceptibility: meropenem (100%), imipenem (100%), piperacillin/tazobactam (96.7%), amikacin (95.6%), and ampicillin/sulbactam (68.7%). Moreover, *P. aeruginosa* was sensitive to ciprofloxacin (73.3% susceptibility), meropenem (69.1% susceptibility), gentamicin (68.4% susceptibility), amikacin (67.1% susceptibility), imipenem (61.2% susceptibility), and ceftazidime (60.8% susceptibility). Overall, Gram-negative bacterial strains exhibited the most resistance to aztreonam, cefotaxime, gentamicin, ciprofloxacin, and sulfamethoxazole in descending order. Furthermore, large quantities of extensively drug-resistant (XDR) strains were detected among *K. pneumoniae, A. baumannii,* and *P. aeruginosa* strains. The resistance rates of the most commonly isolated Gram-negative bacteria, including *K. pneumoniae, P. aeruginosa, A. baumannii,* and *E. coli* to various antimicrobial agents is depicted in **Table 3**.

Resistance Rates of Isolated Pathogenic Bacteria to Common Disinfectants

Prior to the analysis of the resistance of isolated strains to commonly used disinfectants, the MIC and MBC of various disinfectants for standard strain *K. pneumoniae* (ATCC700603) was determined (**Table 4**). The values shown in **Table 4** were therefore defined as the threshold values for the interpretation of resistance of isolated strains to disinfectants.

Compared to antibiotics, clinically isolated pathogenic

Table 3 Antibiotic resistance rates of the four most common Gram-negative bacteria

Antibiotic	Resistance rate (%)			
	Klebsiella pneumoniae	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Escherichia coli*
Piperacillin/tazobactam	44.2	54.6	71.4	3.3
Ampicillin/sulbactam	54.2	44.6	65	31.3
Aztreonam	53.7	51.4	77.1	50
Ceftazidime	53.7	39.2	65	50
Gentamicin	45.9	31.6	67.4	41.4
Amikacin	29.7	32.9	61.4	4.4
Imipenem	23.5	38.8	69.3	0
Meropenem	31.3	30.9	70.7	0
Cefotaxime	50.3	65.3	64	50
Ciprofloxacin	46.7	26.7	70	62.2
Trimethoprim/sulfamethoxazole	26.3	63.5	48.6	63.3

Table 4 MIC and MBC of various disinfectants for standard strains

Disinfectant	Alcohol (μg/mL)	Glutaral (μg/mL)	Chlorhexidine acetate (μg/mL)	Iodine tincture (μg/mL)	Iodophor (μg/mL)	Benzalkonium bromide (μg/mL)	Sodium hypochlorite (μg/mL)
MIC	16	4	16	8	16	8	16
MBC	32	8	32	16	32	16	32

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration

Table 5 Resistance rates of clinically-isolated strains to disinfectants

Strain/disinfectant	Resistance rate (%)						
	Alcohol	Glutaral	Chlorhexidine acetate	Iodine tincture	Iodophor	Benzalkonium bromide	Sodium hypochlorite
Klebsiella pneumoniae	7.63	14.46	12.05	8.84	3.20	6.02	10.10
Pseudomonas aeruginosa	3.90	0	7.80	4.39	5.37	3.41	10.24
Acinetobacter baumannii	10.45	20.90	16.42	8.96	8.96	7.46	0
Escherichia coli	3.33	5.00	1.67	6.67	6.67	3.33	0
Staphylococcus aureus	7.69	15.38	11.54	10.77	10.00	6.92	8.46
Enterococcus	2.86	8.57	2.86	11.43	11.43	8.57	14.29

bacteria showed improved sensitivity to disinfectants and exhibited a generally lower level of resistance (**Table 5**): *K. pneumoniae* had a resistance rate of 14.46% to glutaral, 12.05% to chlorhexidine acetate, and 10.1% to sodium hypochlorite. *P. aeruginosa* exhibited a resistance rate of 10.24% to sodium hypochlorite. *A. baumannii* had a resistance rate of 10.45% to alcohol, 20.90% to glutaral, 16.42% to chlorhexidine acetate, and exhibited the most resistance to disinfectants among all strains. *S. aureus* had a resistance rate of 15.38% to glutaral, 11.54% to chlorhexidine acetate, 10.77% to iodine tincture, and 10.00% to iodophor. *E. coli* had no resistance to sodium hypochlorite and generally exhibited the least resistance to disinfectants among all strains. On average, the isolated strains exhibited the most resistance to glutaral and the most sensitivity to alcohol. See **Table 5** for the resistance rates of clinically isolated pathogenic strains, including 249 strains of *K. pneumoniae*, 205 strains of *P. aeruginosa*, 130 strains of *S. aureus*, 67 strains of *A. baumannii*, 60 strains of *E. coli*, and 35 strains of *Enterococcus*, to alcohol, glutaral, chlorhexidine acetate, iodine tincture, iodophor, benzalkonium bromide, and sodium hypochlorite.

Discussion

In this study, a preliminary understanding of the microecological environment of hospitals in Beijing was captured by examining the resistance of isolated strains of pathogenic bacteria collected from 10 hospitals to antimicrobial agents and commonly used chemical disinfectants. In total, 1,104 strains were collected comprising 74% Gram-negative bacteria, 23% Gram-positive bacteria, and 3% fungi. The most common pathogenic bacteria in clinical infective diseases are *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumannii, Escherichia coli*, and *Enterococcus*, which were also isolated in large quantities from clinical samples in our study. Our results are therefore in agreement with those from the Chinese Bacterial Drug Resistance Monitoring Report of 2014 and 2015[10,11].

Enterobacteriaceae are highly susceptible to carbapenems. Among *Acinetobacter* species, *A. baumannii* exhibited a resistance rate of approximately 80% to carbapenems. In comparison, *P. aeruginosa* was susceptible to ciprofloxacin (73.3%), meropenem (69.1%), gentamicin (68.4%), amikacin (67.1%), imipenem (61.2%), and ceftazidime (60.8%). The susceptibility of *P. aeruginosa* to other drugs was below 60%. Among *K. pneumoniae, A.* baumannii, and *P. aeruginosa*, some XDR strains exhibited a slightly greater resistance rate than in the aforementioned domestic reports. The detection rate of methicillin-resistant strains among Gram-positive cocci and coagulase-negative staphylococci was 46.9% and 47.7%, respectively. No vancomycin- or linezolid-resistant staphylococci were detected, which was similar to the previous domestic drug resistance reports. Among the *Enterococcus* species, *E. faecalis* exhibited a significantly greater susceptibility to the test antimicrobials than *E. faecium*. For both *Enterococcus* spp., vancomycin-resistant strains accounted for 11.6%[10].

The emergence and increase in XDR Gram-negative ba-

cilli poses another great challenge for clinical practice. A delicate balance is necessary, as healthcare professionals must adequately use antibiotics to treat infections, while avoiding the emergence of drug-resistant bacteria. Furthermore, based on the results from drug susceptibility tests, disruption of the route of transmission is particularly important in addition to the proactive treatment of infections in order to curb the transmission of drug-resistant strains within hospitals. Disinfectants with high sensitivity must be used to handle potentially contaminated vectors, such as the hands of healthcare professionals, table surfaces, and medical devices, in the implementation of measures to disrupt the route of transmission. While we observed lower resistance compared with antibiotics, this study demonstrated a certain degree of resistance to multiple chemical disinfectants that are commonly used in clinical practice for elimination of clinically isolated pathogenic microorganisms. Specifically, *Klebsiella pneumoniae* exhibited a resistance rate of 14.46% and 12.05% to glutaral and chlorhexidine acetate, respectively; *Pseudomonas aeruginosa* exhibited a resistance rate of 10.24% to sodium hypochlorite; *Acinetobacter baumannii* exhibited a resistance rate of 10.45%, 20.90%, and 16.42% to alcohol, glutaral, and chlorhexidine acetate, respectively; and *Staphylococcus aureus* had a resistance rate of 15.38%, 11.54%, 10.77%, and 10.00% to glutaral, chlorhexidine acetate, iodine tincture, and iodophor, respectively. On the other hand, *Escherichia coli* showed high susceptibility to various disinfectants. Nonetheless, the resistance rate has increased to varying degrees compared with the domestic monitoring reports of 2012, which is related to continuous extensive use of disinfectants. In addition, this study has also demonstrated that prevalent antimicrobial-resistant strains that consist of antibiotic- and disinfectant-resistant plasmids also exhibit a high resistance rate to disinfectants, in particular *Acinetobacter baumannii*. There is likely some association between these plasmids[12–16]; therefore, future studies that investigate cross-resistance mechanisms are necessary.

Conclusions

This study examined the spectrum for bacterial infective pathogens in Beijing, as well as the status quo of their resistance to antimicrobial agents and common clinical disinfectants. On the basis of our findings, healthcare professionals are advised to consider the supplementation of disinfectants to the standardized use of antimicrobial agents. Furthermore, to prevent bacterial resistance, several disinfectants should be alternated on a regular basis. Long-term storage of disinfectants should be avoided and agents requiring dilutions should be freshly prepared frequently to maintain potency and effectiveness. Finally, comprehensive personal protection measures, in particular enhanced hand hygiene, should be undertaken, which must be practiced prior to and after contact with patients, contaminants, or potentially contaminated surfaces.

Acknowledgements: The authors are grateful to Li Guo and Chunrong Sun for their assistance in patient enrolment and referral, assistance with translation, and preparation of this manuscript. This work was supported by the Technology Plan Program Fund of Dongcheng District [grant number 2014-2-004], Beijing, China.

Conflict of Interest: The authors declare no conflicts of interest.

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(Received, January 11, 2018)
(Accepted, July 6, 2018)