A 4-year surveillance of antimicrobial resistance patterns of Acinetobacter baumanni in a university-affiliated hospital in China

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Acinetobacter baumanni (A. baumannii); antibiotic resistance; carbapenem; nosocomial infection

ABSTRACT

Objectives: To investigate the changes in resistance of *Acinetobacter baumanni* (*A. baumannii*) to different antimicrobial agents and the association of resistance rates with several independent factors: specimen origin, hospital wards, patients' gender and age, from 2008 to 2011.

Methods: Bacterial isolates were isolated from January 2008 to December 2011 in the First Affiliated Hospital of Nanjing Medical University. Antimicrobial susceptibility testing was determined by the Kirby-Bauer Disk Diffusion Agar method as recommended by CLSI. Data were managed by the software WHONET 5.4 and analyzed by SPSS 17.0 software.

Results: The proportion of *A. baumannii* in our hospital increased from 7.0% in 2008 to 18.8% in 2011, becoming the most frequent pathogen in gram-negative isolates. 79.9% of *A. baumannii* were cultured from respiratory tract and geriatrics showed the highest isolation rate of *A. baumannii* during 4 years. The resistance rates to 12 antibiotics significantly increased from 2008 to 2011, from 14.8% to 90.8% to imipenem, and from 23.3% to 91.1% to meropenem. Statistical analysis demonstrated that the specimen origin, hospital wards, patients' gender and age were associated with resistance rate in varying degrees.

Conclusions: The 4-year surveillance of antimicrobial susceptibility demonstrated the rapid increase of drug-resistant strains of *A. baumannii*, and revealed several factors related with resistance rate. Knowing the antimicrobial resistance patterns will help guide the empirical therapy and strengthen interventional infection control measures in China.

KEY WORDS

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Introduction

Acinetobactor baumannii (A. baumannii) is an important opportunistic pathogen causing a wide spectrum of nosocomial infections including pneumonia, bacteremia, surgical-site infections, secondary meningitis, and urinary tract infections, involving mostly patients with impaired host defenses (1,2). In recent years, the increasing resistance rate of *A. baumannii* to a wide range of antibiotics as a result of both intrinsic and acquired mechanisms is causing a serious clinical problem, leading to

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ISSN: 2072-1439 © Pioneer Bioscience Publishing Company. All rights reserved. an increase in morbidity and mortality (3-5). Surveillance is therefore important in providing useful information for physicians in choosing empirical antibiotics. It also helps to address specific resistant issues within a region to identify targeted intervention measures (6,7).

In view of the severity of *A. baumannii* infections and the implications for empirical antibiotic regimens in the setting of high drug resistant strains, we set out to undertake a retrospective analysis of *A. baumannii* isolated in our hospital in 4-year period. The changing trend and associated factors of antimicrobial susceptibility were reviewed.

Materials and methods

Bacterial isolates

The study period spanned from January 2008 to December 2011. Bacterial isolates were collected from outpatients and inpatients in the First Affiliated Hospital of Nanjing Medical University, a tertiary-care hospital with 2,261-bed in China. Only the first

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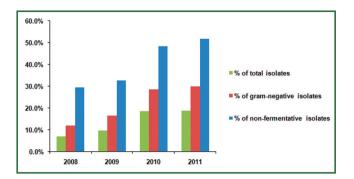


Figure 1. The isolation rate of A. baumannii, 2008-2011.

isolate from each patient was included in this study. All the strains were identified using VITEK-2 (biomérieux, France) and API automated systems (biomérieux, France), supplemented by conventional biochemical tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was determined by the Kirby-Bauer Disk Diffusion Agar method as recommended by Clinical and Laboratory Standards Institute guidelines (CLSI) (8). Bacterial isolation disk diffusion testing was performed on Muller-Hinton agar (Oxoid, UK), using disks (Oxoid) of cephalosporins (cefuroxime, ceftazidime, cefotaxime, cefepime), compound agents (cefperazone/sulbactam, piperacillin/ tazobactam, amoxycillin/clavulanate), carbapenems (imipenem and meropenem), aminoglycosides (amikacin), monobactams (aztreonam), fluoroquinolones (levofloxacin). *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as control strains for susceptibility tests.

Data management and statistical analysis

Data of antimicrobial susceptibility testing were extracted from the laboratory information system and converted centrally into a standard format using WHONET 5.4 (WHO, Switzerland), which was used for data management. SPSS 17.0 software (SPSS Inc., USA) was used for statistical analysis. Results, presented as numbers (percentage) for categorical variables, were compared using Pearson's χ^2 test. For small samples, Fisher's exact test was used to analyze as appropriate. Statistical significance was set at P<0.05.

Results

Changing of A. baumannii isolation rate

From 2008 to 2011, the cases of bacterial infection in the first affiliated hospital of Nanjing medical university were 4,220,

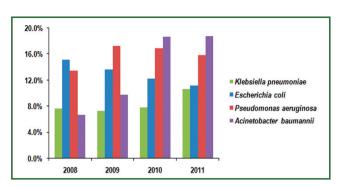


Figure 2. Changing trend of top 4 pathogens in gram-negative isolates, 2008-2011.

5,207, 4,851 and 5,960, respectively. Of these pathogens, there was an overall increase in isolation rate of *A. baumannii*, comprising 7.0%, 9.8%, 18.7% and 18.8% in 2008-2011. The proportion of *A. baumannii* in gram-negative isolates (from 12.0% in 2008 to 29.9% in 2011) and non-fermentative isolates (from 29.6% to 51.9%) also increased year by year (Figure 1). The top 4 frequently isolated pathogens in gram-negative isolates were shown in Figure 2. *Pseudomonas aeruginosa* accounted for 13.5% and 17.2% of all isolates in 2008 and 2009, respectively, while *A. baumannii* became the most common isolated pathogen beyond *Pseudomonas aeruginosa* in gram-negative isolates from 2010 to 2011.

A. baumannii were cultured from respiratory samples (2,256 isolates, 79.7%), followed by blood (113 isolates, 4.0%), catheter (97 isolates, 3.4%), pus (73 isolates, 2.6%), urine (43 isolates, 1.5%) and others (249 isolates, 8.8%) (Figure 3). The main 5 wards that *A. baumannii* was initially isolated were geriatrics (23.0%), emergency department (17.4%), ICU (16.7%), neurosurgery department (9.6%) and respiratory department (8.3%). The changing trend of *A. baumannii* distribution stratified by hospital wards from 2008 to 2011 was shown in Figure 4. The greatest burden of *A. baumannii* infection occurred in geriatric from 2008 to 2011, and only in respiratory department the isolation rate decreased.

Changing trend of antimicrobial susceptibility

Table 1 showed the resistance rate of *A. baumannii* to 12 common antimicrobial agents. In general, our study revealed the continuous increase of antimicrobial resistance to all antibiotics studied from 2008 to 2011, and all the changes were statistically significant (P<0.001).

The changing trend of resistance rate to cefuroxime, amikacin, aztreonam, levofloxacin, imipenem and meropenem over the 4 years was also shown in Figure 5 to highlight the sharp increase of carbapenems resistance rate. Imipenem resistance rates increased from 14.8% in 2008 to 90.8% in 2011, and meropenem from 23.3% in 2008 to 91.1% in 2011. The resistance rates

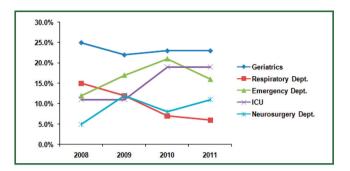


Figure 3. The specimen origin of A. baumannii, 2008-2011.

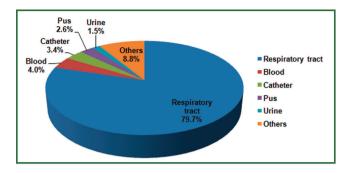


Figure 4. The distribution of *A. baumannii* stratified by hospital wards, 2008-2011.

Table 1. Trends in resistance (R%) of A. baumannii to antibiotics, 2008 to 2011.								
Antibiotics	2008 (n=297)	2009 (n=508)	2010 (n=906)	2011 (n=1,120)	Р			
Cefuroxime	77.8	91.1	95.6	98.5	<0.001			
Ceftazidime	63.7	83.4	93.4	93.6	<0.001			
Cefotaxime	88.2	96.3	98.7	98.4	< 0.001			
Cefepime	51.4	77.4	88.4	92.5	<0.001			
Cefperazone/sulbactam	12.0	44.4	56.0	67.4	< 0.001			
Piperacillin/tazobactam	52.2	81.6	91.6	92.4	<0.001			
Amoxycillin/clavulanate	63.7	85.1	93.9	94.6	< 0.001			
Imipenem	14.8	60.4	85.2	90.8	<0.001			
Meropenem	23.3	59.4	86.0	91.1	< 0.001			
Amikacin	57.9	69.5	74.7	72.3	<0.001			
Aztreonam	72.9	92.5	94.6	97.6	< 0.001			
Levofloxacin	56.8	74.9	79.6	87.3	< 0.001			

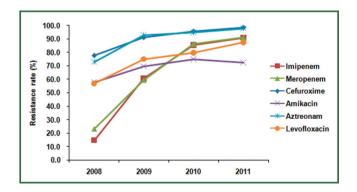


Figure 5. Rapid increase of imipenem and meropenem resistance in *A. baumannii* (P<0.001). The secular trends of resistance to 4 other agents are shown for comparison.

to other agents increased steadily over the years although fluctuation was observed on amikacin (Table 1, Figure 5).

The drug resistance to cephalosporins was also serious during the study period. More than 90% of *A. baumannii* were resistant to cephalosporins in 2011, with 98.5% resistance rate

to cefuroxime and 98.4% resistance rate to cefotaxime. The same increasing trends were also observed in *A. baumannii* against aminoglycosides, monocyclic β -lactams, quinolones and compound agents. The resistance rate of cefperazone/sulbactam also showed a rapid increase from 12.0% in 2008 to 67.4% in 2011.

Antimicrobial susceptibility stratified by specimen origin

Table 2 summarized the resistance rate of *A. baumannii* obtained from 4 sites. The resistance rate to cefepime, meropenem, amikacin and levofloxacin significantly differs among *A. baumannii* from different samples (P-value were 0.031, 0.035, 0.027 and 0.021, respectively). In addition, a relatively lower resistance rate occurred in blood isolates than others, while a higher rate was found in catheter isolates.

Antimicrobial susceptibility stratified by hospital wards

A. baumannii isolated from different wards varied in resistance rate to all antimicrobial agents we studied, and the differences

Table 2. The resistance rate (R%) of A. baumannii to antibiotics stratified by specimen origin.							
Antibiotics	Respiratory tract (n=2,256)	Blood (n=113) Catheter (n=9		Pus (n=73)	Р		
Cefuroxime	94.3	95.5	96.1	91.8	0.736		
Ceftazidime	88.7	87.5	95.8	87.7	0.348		
Cefotaxime	97.3	95.2	100.0	97.3	0.382		
Cefepime	84.6	79.8	94.5	88.0	0.031		
Cefperazone/sulbactam	53.7	56.0	51.8	55.7	0.150		
Piperacillin/tazobactam	86.4	84.2	92.8	87.7	0.623		
Amoxycillin/clavulanate	89.7	87.6	95.9	86.3	0.234		
Imipenem	77.3	70.6	81.2	82.4	0.071		
Meropenem	77.6	67.6	86.9	80.8	0.035		
Amikacin	71.6	63.9	75.3	76.7	0.027		
Aztreonam	87.5	87.5	96.0	90.5	0.207		
Levofloxacin	81.1	68.5	83.9	76.7	0.021		

Table 3. The resistance rate (R%) of to antibiotics stratified by hospital wards.								
Antibiotics	Geriatrics (n=666)	ICU (n=484)	Emergency dept. (n=477)	Neurosurgery dept. (n=265)	Respiratory dept. (n=237)	Р		
Cefuroxime	91.9	97.2	96.6	99.1	91.7	<0.001		
Ceftazidime	86.1	94.9	93.4	97.3	86.8	< 0.001		
Cefotaxime	95.5	99.1	98.9	99.2	97.2	< 0.001		
Cefepime	81.1	92.2	89.8	93.2	81.5	< 0.001		
Cefperazone/sulbactam	61.5	65.8	54.7	47.5	44.4	< 0.001		
Piperacillin/tazobactam	81.3	94.5	91.0	94.6	86.4	< 0.001		
Amoxycillin/clavulanate	88.5	94.0	94.6	96.2	88.2	< 0.001		
Imipenem	75.3	89.1	83.5	81.2	75.6	< 0.001		
Meropenem	76.1	89.2	85.5	77.9	75.2	< 0.001		
Amikacin	65.5	78.4	66.1	86.3	74.7	< 0.001		
Aztreonam	88.5	90.1	91.8	92.4	85.5	0.023		
Levofloxacin	79.4	85.6	84.2	85.9	81.8	<0.001		

were all significant (P<0.001). Worth noting that the highest resistant rate was observed in neurosurgery department, however, in geriatrics and respiratory department the resistance rates were lower (Table 3).

Antimicrobial susceptibility stratified by patients' gender

We divided the patients into male and female group to analyze the difference in the level of drug resistance. As listed in Table 4, the resistance rate to cefperazone/sulbactam, amoxycillin/ clavulanate, imipenem, meropenem and aztreonam were statistically significant between male and female. Interestingly, the resistance rates to these 5 antibiotics in male group were all higher than female.

Antimicrobial susceptibility stratified by patients' age

We divided patients into 10 groups at intervals of ten-year-old to analyze the drug resistance. Data of group 1 (under ten years old) were unavailable for the number was less than 10. The resistance rates to most antibiotics evaluated were statistically different in 9 age groups except meropenem and aztreonam (Table 5). Moreover, the 11-20 and 21-30 group showed higher resistance rates, and the \geq 91 group showed the lowest resistance rates compared with other age groups.

Discussion

A. baumannii is an opportunistic pathogen that is frequently

involved in a variety of infection including pneumonia, septicaemia and urinary tract infection following hospitalization of patients with more severe illness (9). The ability to chronically colonize patients and cause outbreaks which are usually hard to eradicate poses significant challenge to infection control and increases healthcare expenditure (10).

During the study period, the proportion of *A. baumannii* in all bacterial isolates had a dramatic increase, from 7.0% in 2008 to 18.8% in 2011, advancing to the most common pathogen in gram-negative isolates, thus garnered significant attention.

In the present study, nearly 80% of *A. baumannii* recovered from clinical specimens were of respiratory origin. Other sources were blood, catheter, pus and urine. This can be partly explained

Table 4. The resistance rate (\mathbb{R} %) of <i>A. baumannii</i> to antibiotics stratified by patients' gender, 2008 to 2011.							
Antibiotics	Male (n=2,095)	Female (n=693)	Р				
Cefuroxime	94.5	92.7	0.188				
Ceftazidime	89.1	86.7	0.093				
Cefotaxime	97.5	95.8	0.053				
Cefepime	85.1	81.8	0.119				
Cefperazone/sulbactam	56.2	48.5	<0.001				
Piperacillin/tazobactam	86.9	83.2	0.055				
Amoxycillin/clavulanate	90.3	86.6	0.006				
Imipenem	77.9	72.6	0.017				
Meropenem	78.1	72.9	0.017				
Amikacin	70.9	71.7	0.475				
Aztreonam	89.2	84.4	0.001				
Levofloxacin	80.5	78.4	0.109				

by the fact that *A. baumannii*, as the 2th most frequent pathogen causing respiratory tract infection like pneumonia (11), was thus more frequently detected in respiratory sample. In addition, the low examination rate of sterile fluids also resulted in the low isolation rate of *A. baumannii* in our hospital these years. We recommend clinicians to pay more attention to the detection value of *A. baumannii* in sterile fluids, so as to increase the examination rate of body fluids like blood, perfusate and serous effusion.

Our data showed that patients with *A. baumannii* infection mainly came from geriatrics, emergency department, ICU, neurosurgery department and respiratory department. And geriatrics remained the highest isolation rate for 4 years. It may be because patients in these sections were all in critical condition, long duration of hospitalization, lower immune defense, suffering from severe underlying diseases or frequent invasive procedures like tracheotomy. Therefore, patients were in high-risk of hospital-acquired infection (12). In nearly all wards in China, many patients usually resident in one room, so adequate patient separation and infection control measures are important to reduce nosocomial infection.

Our study revealed that there had been a continuous increase in the *in vitro* resistance to 12 antimicrobial agents we evaluated, including cephalosporins, carbapenems, aminoglycoside, quinolones and compound agents. The striking increase in resistance to carbapenems is of particular interest, since this class of antimicrobial agent was, until recently, considered to be among the most potent against many microorganisms including *A. baumannii*. The resistance rates to carbapenems rapidly increased throughout the study period, for imipenem from 14.8% in 2008 to 90.8% in 2011, and for meropenem from 23.3% in 2008 to 91.1% in 2011. The changing trend was much more obvious than other reports showing carbapenemsresistant growth. In Taiwan, the prevalence of imipenem

Table 5. The resistance rate (R%) of A. baumannii to antibiotics stratified by patients' age.										
Antibiotics	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	≥91	Р
	(n=59)	(n=127)	(n=156)	(n=282)	(n=334)	(n=404)	(n=673)	(n=669)	(n=100)	
Cefuroxime	95.7	96.6	89.7	94.4	94.8	97.5	94.1	92.8	85.9	<0.001
Ceftazidime	92.9	89.4	84.2	88.4	91.1	89.2	90.7	86.8	77.1	0.003
Cefotaxime	98.0	95.I	94.5	98.1	98.0	97.4	97.8	96.7	91.9	0.027
Cefepime	92.9	89.8	81.2	81.7	86.5	85.0	84.9	83.8	73.0	0.015
Cefperazone/sulbactam	69.0	57. I	45.8	53.4	50.5	46.6	53.8	61.1	60.2	<0.001
Piperacillin/tazobactam	91.1	87.9	81.5	85.2	88.5	87.7	87.8	83.7	76.8	0.009
Amoxycillin/clavulanate	92.7	93.7	83.2	88.3	91.4	91.1	90.0	89.1	81.6	0.001
Imipenem	76.4	82.8	70.7	75.3	76.7	74.6	80.1	75.6	72.3	0.032
Meropenem	78.2	82.4	72.1	74.7	74.8	75.4	80.2	76.7	72.0	0.064
Amikacin	76.5	78.9	70.0	66.8	74.8	73.4	73.0	66.0	69.1	0.016
Aztreonam	92.9	88.8	85.6	88.6	85.4	91.0	87.6	88.4	81.4	0.111
Levofloxacin	74.I	78.8	74.7	75.7	82.1	80.3	80.8	84.2	64.1	< 0.001

resistance over 10 years was from 3.4% in 2002 to 58.7% in 2010 (13). A SENTRY study also reported that resistance to imipenem changed from 34.5% in 2006 to 59.8% in 2009 overall worldwide (14). The major mechanism of carbapenems resistance in *A. baumannii* is the production of carbapenemases including Ambler class B metallo- β -lactamases (MBLs) and the carbapenem-hydrolyzing class D β -lactamases (CHDLs), also called oxacillinases (OXAs), with the latter more frequently in *A. baumannii*. Several studies in China have showed that $bla_{oxa-23-like}$ gene, perfectly associated with carbapenem resistance, could be used to predict imipenem resistance of *A. baumannii* (15,16).

The increasing emergent of highly aminoglycosides-resistant strains caused a major concern. Our result on resistance rate to amikacin was 94.6% in 2011 higher than 34% in Latin America reported by a SENTRY antimicrobial surveillance program (17). Moreover, the resistance rates to β -lactams antibiotics such as third/fourth-generation cephalosporins or compound agents increased gradually, especially for cefperazone/sulbactam, from 12.0% in 2008 to 67.4% in 2011.

It seems that our resistance rates of *A. baumannii* to antibiotics were generally much higher than data from other regions. The difference could likely be due to inappropriate, uncontrolled empiric therapy or cross acquisition of resistance in our hospital. These reasons highlight the urgent need for supervising antibiotic use and strengthening infection control measures in our hospital. New therapeutic strategy should be introduced in clinical practice for the treatment of multi-resistant *A. baumannii* infection. Recent observations had shown that combination antibiotic therapy (e.g., polymyxin B combined with imipenem, imipenem plus rifampin, or the triple combination of poly-myxin B, imipenem and rifampin) was synergistic when tested against imipenem-resistant strains (18).

Although increasing resistance rate of *A. baumannii* worldwide has been reported before (19,20), few studies reported antimicrobial susceptibility patterns affected by several independent factors: specimen origin, hospital wards, patients' gender and age.

As to sample origin, the resistance rate to cefepime, meropenem, amikacin and levofloxacin significantly differs. The isolates from blood showed the lowest resistance rate. In contrast, the highest resistance rate was found in catheter, which may be related to nosocomial infection. At least 37.5% of all nosocomial infections were due to cross-transmissions of colonized pathogen between patients, thus increase the risk of drug-resistance (21). Although *A. baumannii* was mostly detected in respiratory samples, the resistance rate was relatively not so high. Sample size might be the reason, which was 2,256 from respiratory tract, twenty-fold greater than others.

Data concerning the resistance rate of *A. baumannii* from patients receiving care in different wards were also analyzed. In geriatrics and respiratory department, the resistance rates of *A.*

baumannii were much lower. Of interest, the highest resistance rate was found in patients from neurosurgery department rather than ICU as other studies reported (22,23). Characteristics of neurosurgery, such as a higher proportion of patients undergoing neurosurgical surgery, a more frequent use of invasive procedures like intraventricular catheters, and a more illegitimate antibiotics use because of the unremarkable clinical manifestations of post-surgical infections, compared to other hospital wards, may cause the result (24).

We analyzed the resistance rates according to patients' gender and age. The statistically difference was only found in 5 antibiotics between male and female, but it is still noteworthy that the resistance rate in male group were all higher than female in these 5 antibiotics. To our knowledge, variation in resistance rate profiles among different age groups has rarely been addressed. Our study revealed that the resistance rates to most of antibiotics were statistically significant among 9 age groups. The 11-20 and 21-30 age groups showed higher resistance rates compared to other age groups. The lowest resistance rate was in \geq 91 age group, which was coincidently in agreement with the lower resistance rate in geriatrics. It seems that elderly patients (≥ 91) were more susceptible to antimicrobial agents during the therapy of A. baumannii infections. Whether the lower drugresistance of isolates from elderly patients resulted from the rational use of antibiotics or less frequency of clonal transmission remained to be elucidated.

It should be acknowledged that our data were retrieved from only one hospital, so continuous multi-center surveillance of antimicrobial susceptibility of *A. baumannii* in China is still necessary to generate enough representative data. Moreover, molecular typing analysis, such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and drug-resistance gene typing (DRGT), should be performed. Thus, existing outbreaks caused by epidemic clonal spread of a single resistant strain could be monitored.

In conclusion, this study investigated antimicrobial susceptibility of *A. baumannii* and its related influencing factors in a university-affiliated hospital in China during 4 years. Knowing the rapidly increased resistance rate of *A. baumannii* and the association with specimen origin, hospital wards, patients' gender and age, is important to better understanding the antimicrobial patterns, and optimize antimicrobial prescription policies to control the occurrence of drug-resistant *A. baumannii*.

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