Original Article

Drug Resistance of Acinetobacter in Selected Hospitals

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Abstract

Background: Nowadays, nosocomial infection with multidrug-resistant Acinetobacter is an important problem in the world, which is facing wide spectrum antibiotics and hence has become resistant.

Materials and Methods: In this study, positive cultures of Acinetobacter from one hundred clinical samples in seven hospitals from Tehran during 2012-2013 were collected for checking antibiotic susceptibility. Samples test with Ceftazidim, Cefepime, Amikacine and Imipenem by E-test and for Tazocin, Colistin and Tigecycline was performed with disk diffusion method.

Results: For Colistin 10 samples, and for Tazocin, 40 samples were performed by E-test method. Then *boumannii* species of bacteria and non-*baumannii* Acinetobacter were separated by PCR and antibiotic susceptibility testing was performed on them. 89% of Acinetobacter samples were *boumannii* species, which was isolated from respiratory secretions at ICU.

Conclusion: Boumannii and non-boumannii species of bacteria with a high percentage were resistant to Ceftazidim, Amikacine, Cefepime, Tazocin and Imipenem. All baumannii and non-boumannii Acinetobacter were sensitive to Colistin, were only 75% sensitive to Tigecycline, which is a new glycylcycline. Colistin and Tazocin results in samples limited to the E-test method were similar with disk diffusion.

Keywords: Acinetobacter, Drug Resistance

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Introduction

Nosocomial infections are one of the health problems of modern societies, which are rising with unusual organisms. Acinetobacter, which is the main cause of nosocomial infections such as pneumonia and nosocomial pneumonia, is caused by mechanical ventilation. Acinetobacter species are becoming resistant to antibiotics. Acinetobacter formed 4% of cases of pneumonia in the intensive care unit (ICU) in 1986, 6% in 2003 and 7% in the years 1992 -1997 in the United States and the rate is still increasing[1].

Resistant cases of Acinetobacter *baumannii* in the neonatal ICU[2], and cases of osteomyelitis caused by multidrug-resistant Acinetobacter in Iraq have been reported[3].

Overflow Acinetobacter infection during 1977-2000 occurred in Palestine. During the 25 years before outbreak Acinetobacter resistance to antibiotics such as Aminopenicillins, Ureidopenicillin, first-generation Cephalosporins, Cephamycin, and most of the aminoglycosides and tetracycline was reported[4]. Acinetobacter prevalence of multidrug-resistant, which was 1.2% in 2002, reached to 9.7% in 2006[5] and at polyclonal genotype, the emergence of resistant strains in Israel during the years 2003-2008 is reported[6].

Acinetobacter outbreak in hospitals was mainly caused

by items such as respiratory care, water source of moisturizers and wound lavage system[7].

The main site of infection with this pathogen, like other Gram-negative, is lower respiratory tract and urinary tract. Identification of Acinetobacter in the ICU is difficult since this pathogen has the ability of colonization, especially in skin, throat, and temporarily in tracheostomy patients[8].

Acinetobacter infections, mostly progress towards bacteremia, septicemia also lower respiratory tract involvement with Acinetobacter, are the most common source of infection in regards to Acinetobacter progression towards bacteremia and septicemia[9].

Taking into consideration the importance of the resistant Acinetobacter in nosocomial infection and overflow appearance of this kind of infections in various hospitals, health centers and in different periods of times and since Acinetobacter infection with antibiotic-resistant is important factor of hospital mortality, increased length of hospitalization, extra costs which in turn has adverse emotional, psychological effects on patients and their companions, health budget and has adverse are created. We intend to identify resistant Acinetobacter and antibiotic resistance hence taking a positive step towards improving healthcare status, the quality of treatment and practitioners' knowledge about correct and timely use of antibiotics, in order to avoid creating more resistance species also we want to review antibiotic susceptibility status in Iran (Tehran) review.

Methods

This study has been a cross series survey which has been done on 100 samples during 2012-2013 periods. Our samples were patients admitted to the ICU, surgical, pediatric and etc. wards in Khatamolanbya, Parsian, Iranmehr, Milad, Loghman Hakim and Labbafinejad hospitals by observing the entry and exit rules of this study.

After receiving permission from the authorities of hospitals and laboratories, the samples of blood, urine, sputum and tracheal tube were collected from patients needed to be cultured and were sent to the hospital laboratories. Then after providing direct smear from the samples, they were taken to the culture medium immediately and were kept in the incubator. For culturing the clinical samples, Blood agar and EMB agar mediums were used. Grown Gram-negative samples were tested on the basis of certain characteristics of Acinetobacter pathogens. Through examination of oxidase activity (negative oxidase), the ability to move the germs in the SH2 and DAPI (without movement), growth in citrate (no growth), colonies form in the TSI (Triple Sugar Iron), samples were found positive for Acintobacter. These samples were sent to reference Laboratories (Milad hospital's Microbiology Laboratory, Infectious Disease Research Center of Labbafinejad hospital) for susceptibility and antibiotic resistance examination. Also Acinetobacter samples were isolated from baumannii strains by PCR method.

Antibiotic susceptibility and resistance of Ceftazidime, Cefepime, Amikacin, Imipenem antibiotics were examined by E-test method through antibiotic strips of Zistmand company, and Tigecycline, Colistin and Tazocin antibiotics were investigated by disk diffusion method (since antibiotical strips were not available) though antibiotical disks of mast or oxoid company. 10% of samples for Colistin and 40% of samples for Tazosin were examined by both E test and disk diffusion methods.

Acinetobacter Blood agar colonies were transferred by MAC method and standard, with 0.5 opacity McFarland standard to broth culture medium. Then Acinetobacter colonies were transferred from Antibiogram culture to Mueller Hinton agar medium. Later Ceftazidime, Cefepime, Amikacin, Imipenem antibiotical strips were kept in MH agar medium at 35°C for overnight (about 18 hours), and then antibiogram and susceptibility to antibiotics results were obtained from degrees and MIC's on antibiotical strips. For Tigocycline and Tazocin antibiotics, the antibiotical discs were kept in MH agar medium for 18-24 hours at 35°C then antibiogram results were obtained by disk diffusion methods and using special tables on the bases of CLSI standard. After obtaining Acintobacter antibiotical susceptibility and resistance results we used SPSS software and descriptive statistical methods to analyses these results.

Results

Of 100 Acinetobacter samples which were collected

Table 1: The range of MIC antibiotics in E-test according to CLSI standards.

Antibiotics	S	I	R
Ceftazidim	<0.5	0.5-2	>2
Cefepime	<1	1-4	>4
Imipenem	<1	1-4	>4
Tazocin	<1	1-8	>8

Table 2: Antimicrobial susceptibility testing by disk diffusion range (CLSI standard).

Antibiotics	S	I	R
Tazocin	>16mm	13-15mm	<12mm
Imipenem	>21mm	18-20mm	<17mm
Colistin	>16mm	14-15mm	<13mm
Tigecycline	>11mm	-	<11mm

Table 3: Antibiotic susceptibility of Acinetobacter baumannii strains by E-test and disk diffusion methods.

Antibiotics	Resistant	Sensitive	Intermediate
ceftazidim	100	0	0
cefepime	98	2	0
imipenem	77	12	11
amikacine	100	0	0
tazocin	84	14	2
colistin	0	100	0
tigecycline	4	76	20

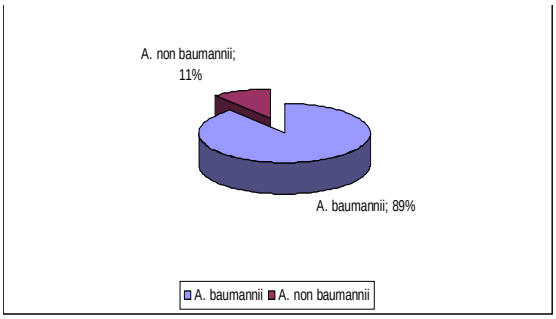

Figure 1. Distribution of Acinetobacter by species

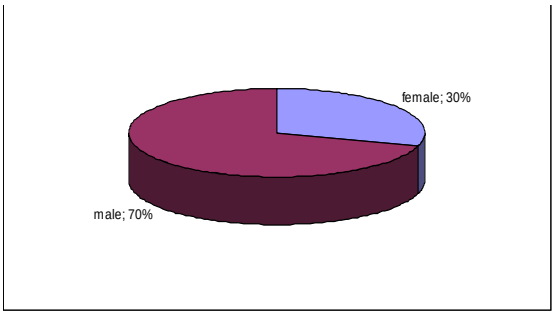

Figure 2. Distribution of Acinetobacter by sample's sex

within 6 months from seven hospitals in Tehran, we were able to get samples of respiratory secretions, wounds, catheters, urine, CSF, brain abscess. Besides we collected positive cultured samples of Acinetobacter from ICU, Burn, surgery, internal medicine, and neurology wards. Out of 100 samples we had only one case from one-year-old child. Most of the patients were male in age range of 1 to 87 years old with an average of 46.2 years. The age distribution did not differ between males and females. Highest number of samples was from respiratory secretions and the least of them brain abscess (only one sample).

Most of the Acinetobacter samples were isolated from ICU. The age average of patients in the ICU and the internal ward was 60 years and in the burn unit was 27 years. Sample distributions based on Acinetobacter species according to gender variable, clinical specimens, and hospital wards are shown in figures 1-6.

To investigate antibiotic susceptibility of Imipenem, Ceftazidime, Cefepime Amikacin antibiotics we used the E-test method and disk diffusion test for Colistin, Tazocin and Tigecycline antibiotics. Tigecycline and

Table 4: Antibiotic susceptibility of Acinetobacter non- baumannii strains by E-test and disk diffusion methods.

Antibiotics	Resistant	Sensitive	Intermediate
ceftazidim	100	0	0
cefepime	91	9	0
imipenem	64	18	11
amikacine	100	0	0
tazocin	82	9	2
colistin	0	100	0
tigecycline	0	73	27

Table 5: Antibiotic susceptibility of Acinetobacter species generally by E-test and disk diffusion.

Antibiotics	resistant	Sensitive	Intermediate
ceftazidim	100	0	0
cefepime	94.5	5.5	0
imipenem	64	15	14.5
amikacine	100	0	0
tazocin	83	11.5	5.5
colistin	0	100	0
tigecycline	2	74.5	23.5

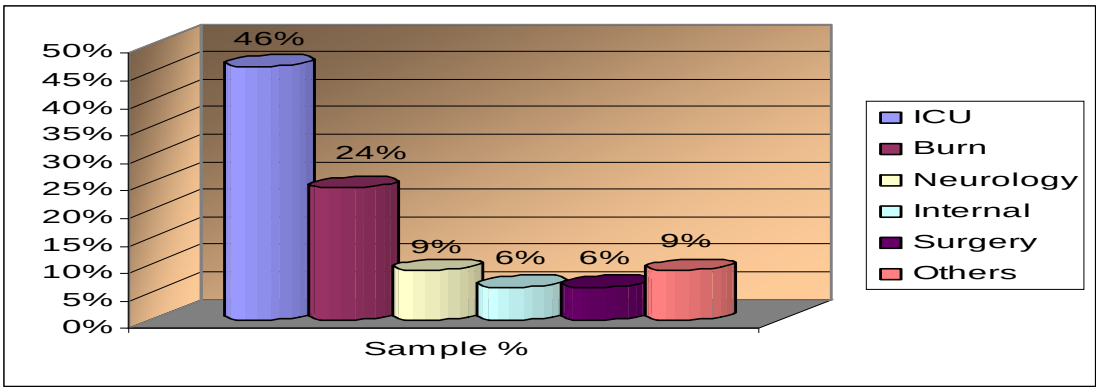

Figure 3. Typical Distribution of Acinetobacter baumannii in hospital wards

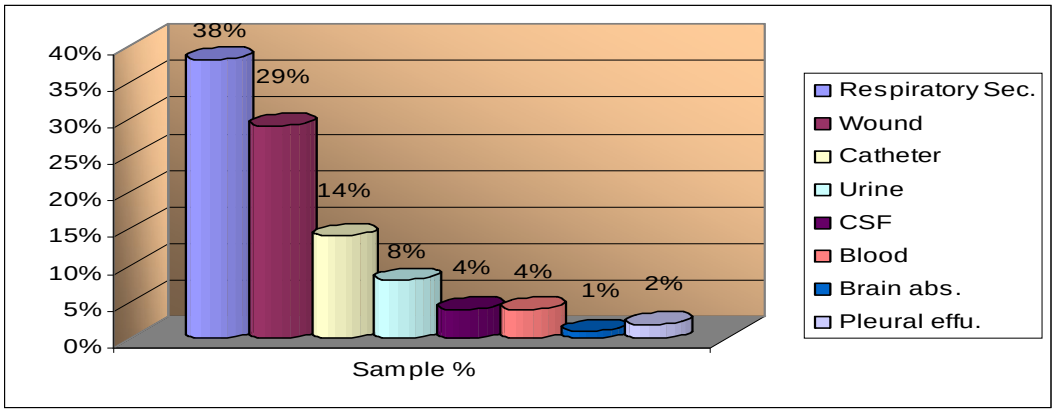

Figure 4. Distribution of Acinetobacter baumannii clinical samples

Colistin were examined by MIC range of E-test and antibiotical susceptibility range of disk diffusion

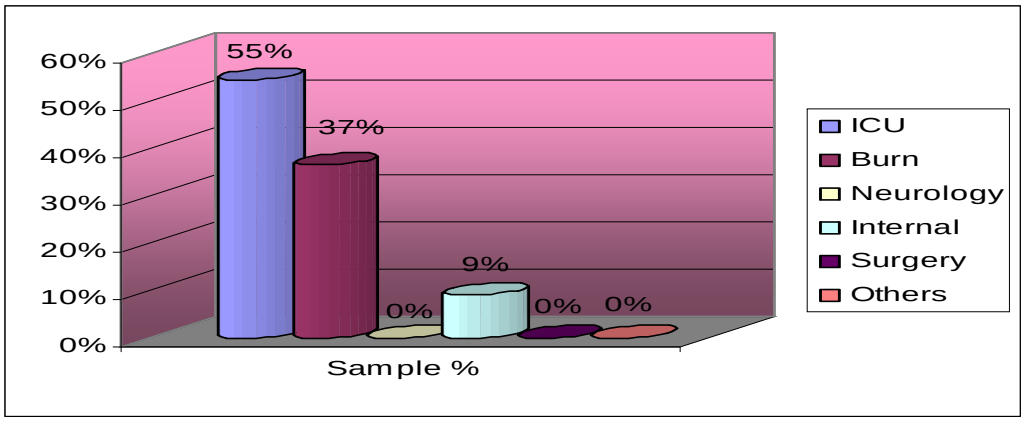

Figure 5. Distribution of Acinetobacter non-baumannii example in hospital wards

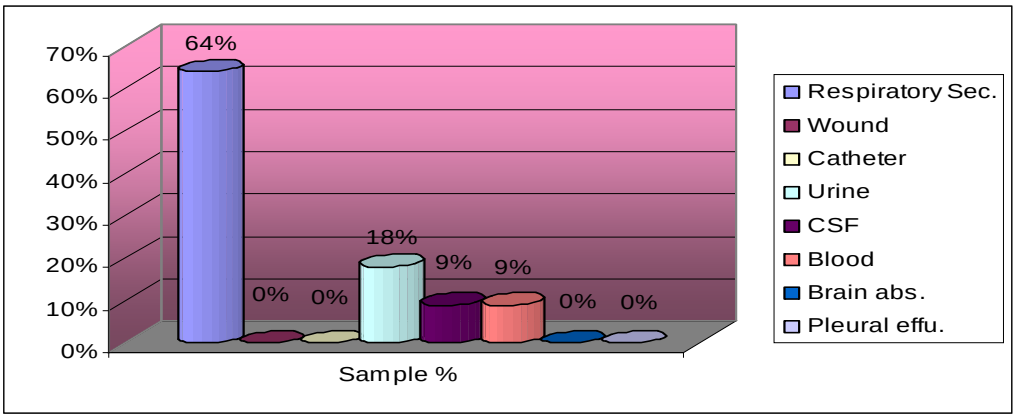

Figure 6. Distribution of non-clinical samples of Acintobacter baumanni

method, which the related results are shown in tables 1-2. Out Of 100 Acinetobacter samples, 40 samples for Tazocin and 10 samples for colistin were done by E-test method. The results were 80% resistance for Tazocin (84% in the disk diffusion method) and 100% sensitivity in Colistin case (100% sensitive in the disk diffusion method). The results of Antibiotical susceptibility of Acinetobacter *baumannii* and non-baumannii, gained from using E-test for Ceftazidime, Cefepime, Imipenem and Amikacin and using the disk diffusion method for Tazocin, Colistin and Tigecycline antibiotics are shown in the tables 3-5.

Discussion

Today, in spite of increasing survival rate of patients due to advances in medical technology and better conditions of intensive care in the ICUs, we are facing with the problem of the emergence of pathogens resistant to several antibiotics and hospital infections. One of these problems is Acintobacter, which its infection in hospitals especially in ICUs has been reported in many countries[2,6]. Inappropriate prescription of highly used antibiotics for treatment of infections, has caused the emergence of multi-drug resistant pathogens including Acinetobacter, hence research results show the increasing resistance of Acinetobacter to highly used antibiotics[29]. Today we are facing with the problem of dominance of Acinetobacter *baumannii*[14] which our study also reached to the same result. Same as other studies our research showed that Acinetobacter has the most relation with respiratory system and most of the positive samples for Acinetobacters were obtained from respiratory secretions and the respiratory tract is the main source of Acinetobacter infection[4,6,8]. As with other studies, our study also reported the most positive culture Acinetobacter from the ICU. Factors such as longer residency of patients in ICU, the need to respiratory protection devices, catheters, and the

underlying disease conditions of patients have provided the possibility of resistant pathogens[7]. Most of the studies have analyzed the antibiotic sensitivity of Acinetobacter by measuring the MIC with Micro dilution broth method. Some studies have used E-test method to analyze antibiotic susceptibility to Tigecycline and Colistin[19,22].

Although other studies, which have been done in the years 1999-2001 and 2006, show the increasing resistance acinetobacter to Beta-lactam, Carbapenem and Amino-glycosides antibiotics, Acinetobacter has retained its sensitivity to Colistin[12]. Of course we do have some reports of heteroresistancy to Colistin among Acinetobacter strains[17]. Besides, genotypes of *Acinetobacter baumannii* resistant to Colistin and Polymyxin B have been identified[21]. Resistance to Colistin among enterobacteriaceae has been reported mostly for Stenotrophomonas maltophilia species and lower degree in *Pseudomonas aeruginosa* rather than in Acinetobacter[13]. In our study, the results of antibiotic sensitivity in *Acinetobacter baumannii* to Ceftazidime, Cefepime, Tazocin, Imipenem and Colistin antibiotics has been similar to the results of a study which has been done in Italy during 2004-2005. In that study, 97.5% to Ceftazidime, 96.2% to Cefepime, 90% to Tazocin, 77.5% to Imipenem, 3.7% to Tigecycline and 1.2% to Colistin[18]. Our results have been also similar to the study, which has been done on antibiotical sensitivity to *Acinetobacter baumannii* with MIC method in Brooklyn, USA 2006. In this study, 85% to Ceftazidime, Cefepime 89%, Tazocin 80%, 63% to Imipenem and 3% to Colistin[12]. The result of another study on antibiotic sensitivity to *Acinetobacter baumannii* in Tehran during 2006-2005, which was performed by disk diffusion method, has been relatively similar to the results of our study. In that study, the *Acinetobacter bumanii*'s resistance has been 95.3% to Ceftazidime, 62% Tazocin and 50.9% to Imipenem[24].

The results on antibiotical susceptibility for Ceftazidime, Cefepime and Tazocin in our study have been similar to the survey, which has been done on *Acinetobacter baumannii* in Iraq and Kuwait during 2003-2004. (The study was carried out with MIC method antibiotical sensitivity has been 83% to Ceftazidime, 78% Cefepime and 89% to Tazocin. But in that survey, antibiotical sensitivity to Imipenem has been 90%, which has significant difference with the results of our study[16]. In a study in Taiwan during the 1997-2001 Acinetobacter species has been 90.6% sensitive to Imipenem[11], but in 2005, in Columbia, Acinetobacter resistant to Carbapenem with OXA-23 and OXA-51 genes were identified[10]. The different results obtained in these studies are due to several factors including time, climatic conditions, type of antibiotics used in that community and the underlying condition of patients. We also should consider mutations, which is happening in *Acinetobacter baumannii* gene and making it resistant to Carbapenem.

The results of our study on antibiotical sensitivity of *Acinetobacter baumannii* to Amikacin have significant difference with the other studies[12,16,18,24]. In a study done in Australia, bactericidal effect of Amikacin on sensitive and resistant Acintobacters to Colistin has been satisfactory[15] which are in contradiction with our results. The result of this study has been only consistent with the survey done in Ahvaz (south west of Iran) by disk diffusion method in 2003-2004 which Acinetobacter was 100% resistance to Amikasin[25]. The reason for such a different result may perhaps be due to paitents' underlying factors and their recent consumption of Amikasin.

In our study, Antibiotical sensitivity in the case of Colistin has been consistent with other studies except the cases, which heteroresistancy to Colistin has been reported, and studies, which have described *Acinetobacter baumannii* species resistance to Colistin from genetic point of view[12,14,16,18,21,23].

The results on Colistin and Tazocin on limited samples, which have been performed by disk diffusion method and E-test method, have not been consistent. In E-test method (40 samples) 80% were resistant to Tazocin and in disk diffusion method 84% resistance to Tazocin were observed. All 10 samples, which were done by E-test method for Colistin, those were sensitive to it.

In studies, which have been done on the correlation between E-test and MIC results on Colistin they concluded, that at lower levels of MIC the correlation exists but for higher quantity of MIC the correlation did not be confirmed[22]. The results of our study on *Acinetobacter baumannii* antibiotic sensitivity to Tigecycline which was obtained by disk diffusion

method were to some extend similar to the results of Korean study for period 2004-2006(56% sensitivity), and with the report Journal of Antimicrobial Chemotherapy (JAC) in April 2007 (78% sensitive), and study done in Rome, Italy during 2004-2005 (5/96% sensitive).

It should be mentioned that in the said studies the antibiotic sensitivity were done by MIC method [13,18,24].

Although in the global program for survey of Acinetobacter species sensitivity to Tigecycline, some cases for high resistance to Tigecycline in Argentina has been reported. But it may be possible that overexpression of intrinsic multidrug efflux pump has reduced the susceptibility to Tigecyclin among Acinetobacter species[19].

In studies, which were done on the correlation between E-test and MIC results for Tigecycline, the results showed a week correlation. This weak correlation was more obvious in species with higher resistance[20].

Differences in antibiotic sensitivity results of our study with other studies are due to the patient underlying factors, consumption pattern of antibiotics, in the region, antibiotic resistance pattern in the region, and also the test methods. It should be mentioned that the Prescription pattern and dose of antibiotics used in the treatment *Acinetobacter baumannii* have important rules in the results obtained.

And also as was resulted in a study done on VAP, in lung transplant patients, it is necessary to use Cefepime and Tigecycline high-dose intravenous in treatment of *Acinetobacter baumannii* infection[29].

In our study, there were no significant differences in the results of *Acinetobacter baumannii* with non-baumannii. In comparison with other studies, in our study we observed increasing trend in antibiotic resistance for Imipenem, Amikacin and Tazocin.

Antibiotic resistance to Imipenem, which was 3% in the study done in England in 2000, and 40% in the study done in Tehran during 2005-2006, was 64% in our study [14,24].

Antibiotic resistance to Tazocin, which was about 5% in the study done in England in 2000, and 25% in the study done in Tehran during 2005-2006, was 82% in our study. About Amicasin resistance, which was 18% in the study done in England in 2000, 45% in the study done in Tehran during 2005-2006, was 100 % in our study.

Ceftazidime resistance, which was 73% in the 2000 British study, 70% in the study done in Tehran during 2005-2006, 100% in our study[14,24].

Due to high percentage resistance of baumannii and non-baumannii strains to most antibiotics except Colistin and Tigecycline, there were no significant differences in the analysis of antibiotic sensitivity condition in respect to sample type or sample collection place, also it did not have any effect on decision making about a type of antibiotic for different infections.

There were some constraints in our study such as the impossibility of infection differentiation from colonization also due to 6 month time period for collecting samples and repeated passages on the samples it is possible that the conditions have been provided for more mutations in Acinetobacter species and their higher resistance to antibiotics.

Another constrain was lack of E-test Strip for Tigecycline, and limited number of E-test steeps for Tazocin and Colistin in Iran which had made the compression of the results of our study with other studies difficult. So it is necessary to adjust the tariffs to reduce the costs of needed materials. Besides we should try through differentiation Acinetobacter infection from colonization avoiding unnecessary treatments and retaining Acintobacter susceptibility to Colistin and Tigecycline in our region.

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