Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at www.cibtech.org/sp.ed/jls/2015/01/jls.htm
2015 Vol.5 (S1), pp. 5592-5597/Salehniya et al.
Research Article

A SURVEY ON FREQUENCY AND ANTIBIOTIC RESISTANCE OF *ACINETOBACTER BAUMANNI* ISOLATED FROM INPATIENTS IN IRAN HOSPITALS DURING 2013-2014

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ABSTRACT
Acinetobacter is a gram-negative cocobacillus. The prevalence of nosocomial infections caused by these bacteria has been on the rise over the last decade. This is especially of more importance in patients admitted to intensive care units. The present study aimed at studying the frequency and also antibiotic sensitivity and resistance of *A.baumanni* and *A.lwoffi* as opportunistic pathogenic agents in Iran hospitals. During this study, over a one-year period, 140 samples of patients admitted to different parts of the hospital were collected and isolates were identified by biochemical tests. Then, antibiogram test was done using Mueller Hinton agar medium and 10 antibiotic discs in order to investigate antibiotic sensitivity and resistance of the bacteria. Results were reported according to CLSI. The highest sensitivities of *A.baumanni* were to the antibiotics ciprofloxacin and ceftriaxone with the frequency of 49% and 48%, respectively; the lowest sensitivities of this bacterium were to tetracycline and erythromycin with 15% and 17%, respectively. *A.baumanni* strains with multi-resistance are regarded as a growing problem for medical centers around the world. Also, the prevalence of these bacteria is increasing in hospitals, so the arbitrary use of antibiotics should be seriously avoided.

Keywords: *A.baumanni, A.lwoffi, Nosocomial Infections, Antibiotic Resistance*

INTRODUCTION
Acinetobacter spp. are gram-negative cocci or cocobacilli with no fermentation power. These bacteria have little nutritional requirements for growth and can survive for a long time in unfavorable conditions, dry surfaces and also an aqueous environment. Although this bacterium is usually of low virulence, it causes a wide variety of infections through the equipment associated with the respiratory systems and infected catheters. The main problem in its infections results from its resistance to beta-lactam antibiotics (David *et al.,* 2006). *A.baumanni* infection in hospitals involves respiratory tracts in a relatively prevalent way. Moreover, *A.baumanni* can cause nosocomial infections of urinary tract and ulcers, and infections may also develop into septicemia. The bacteria are highly resistant to antimicrobial agents and this resistance can be innate or through obtaining resistance genetic factors. Most *A.baumanni* strains are resistant to Ampicillin, Amoxicillin-Clavulanic acid, anti-staphylococcal penicillin, Cephalosporins with a wide range (except for Ceftazidime and Cefepime), tetracycline, macrolides, Rifampin and Chloramphenicol. *A.baumanni* colonization level is growing in inpatients, especially in those with long hospitalization or those who have received a broad antimicrobial treatment or an anticancer treatment (Wareham, 2008). In recent years, *A.baumanni* has been known as one of the important pathogens responsible for severe infections and epidemics of nosocomial infections, particularly in elderly patients, patients with immune disorders, patients admitted to ICU and patients undergoing surgery. Some reasons are the natural tendency of this organism to gain resistance indices, its unnatural ability to survive for a long time in hospital environment and, as a result, its broad antimicrobial resistance to cause nosocomial outbreaks, especially multisystem infection. Hospital-acquired pneumonia is still considered to be the most dominant disease caused by this organism. However, various infections such as bacteremia, septicemia, endocarditis, surgical site infection, urinary tract infection, meningitis, central nervous system infection, and soft tissue have also been reported in recent years. Treatment of infections caused by multi-resistant *Acinetobacter* has become a global problem and a major concern (Valencia *et al.,* 2009).

MATERIALS AND METHODS

Sampling

In this study, 120 samples from among inpatients and 20 samples from different parts of Anzali hospital in Iran were collected. Clinical samples included respiratory secretions (30 samples), urine and urinary catheters (40 samples), wounds (35 samples), and blood (15 samples). Environmental samples included the surfaces of the hospital's different devices (5 samples) and beds and floor (15 samples). Among 120 clinical samples, 70 were related to women and 50 were related to men. The patients were in the 16-90 age groups.

Detection and Isolation Method

For blood culture from patients asking it, 5 mL of blood samples was inoculated in Trypticase Soy Broth and incubated at 37°C for 7-10 days. After each period of 1-2 days, the media were again harvested and cultured in the media MacConkey agar and blood agar. For wound samples, adequate samples were taken for culturing by a sterile swab and by cleaning the wound site with sterile physiologic serum around the samples opened with a scalpel. Then, the above samples were immediately cultured in the media MacConkey agar, blood agar, and brain-heart infusion agar. From urine samples of the patients suspected of urinary infection, negative in terms of other routine factors, and without any antibiotic treatment, counting and culturing was done for morning urine sediment samples following centrifugation in MacConkey, blood agar, and BHIA media. Also, junction of the catheter which was being used for patients for a long time was cut out for 5 cm using sterile forceps. In order to sample lower respiratory secretions (sputum) or the aspirate samples collected by a specialist during bronchoscopy or bronchial washing in the above media. In the present research, as mentioned before, 20 different environmental samples were gathered so that various devices and equipment as well as different surfaces and floors of the hospital were sampled using a sterile swab during peak times and before washing and disinfecting the hospital environment and were cultured in the above media after 4-6 hours of inoculation in BHIB culture. Finally, gram staining was performed for all samples colonized in media cultures and suspected of *Acinetobacter* with biochemical tests.

Antibiogram Test

In order to determine antibiotic sensitivity pattern from confirmed bacterial isolates of *A.baumanni* and *A.lowfii*, we used disc diffusion method in agar (Kirby-Bauer) in Mueller Hinton Agar the product of German Merck Company according to CLSI guidelines. In this test, discs containing the antibiotics Gentamicin(10 µg), Kanamycin(30 µg), Ciprofloxacin(5 µg), Ceftriaxone(30 µg), Sulfamethoxazole(10 µg), Cefotaxime(30 µg), Piperacillin(100 µg), Ampicillin (10 µg), Tetracycline(30 µg) and Erythromycin(15 µg) were used.

RESULTS AND DISCUSSION

Results

A.baumanni isolates grew well on the media used and their colonies size was 2-3 mm in diameter after 24-48 hours of incubation. This bacterium also showed the production of light yellow to grayish with pigments; however, it had the ability to produce pigments in blood agar medium. In contrast, *A.lowfii* species were unpigmented on normal media and produced smaller colonies. *A.baumanni* possessed a high metabolic activity so that it could produce acid from most carbohydrates, but acid production from maltose and urea hydrolysis was varied. All strains were positive for citrate test but negative for acid production from mannitol and sucrose, esculin hydrolysis, SH2 production in TSI medium, nitrate reduction reaction, and MR and VP tests. But *A.lowfii* species had a limited metabolic activity in such a way that showed a positive catalase test but negative reactions in the majority of substrates in use.

In this research, from among 120 clinical samples, 23 *A.baumanni* and 4 *A.lowfi w*ere isolated. Of the 30 samples related to respiratory secretions and sputum, 40 samples related to urine and urinary catheters, 15 wound samples, and 15 blood samples, 9, 7, 4, and 3 *A.baumanni* cases were isolated, respectively, and the number of *A.lowfii* cases isolated from every one of the above samples was only one. Moreover, 5 samples from the surfaces of different devices and 15 samples from the surfaces, beds and, and floors of

the hospital were collected. Among environmental samples, *A.baumanni* had a frequency of 8 and *A.lowfii* had a frequency of 2 cases. Maximum sensitivity of *A.baumanni* was to the antibiotics ciprofloxacin and ceftriaxone with the amounts of 49% and 48%, and its minimum sensitivity was to the antibiotics tetracycline and erythromycin with the amounts of 15% and 17%, respectively. Additionally, its resistance to the antibiotics Cefotaxime and Gentamicin was 52% and 54%, respectively.

The highest *A.lowfii* sensitivity was to the antibiotics Kanamycin, Ciprofloxacin, and Ceftriaxone with 83.33% for each and, secondly, to Gentamicin and Cefotaxime with 66.68% for each. However, this bacterium is equally resistant to the antibiotics Gentamicin, Kanamycin, ciprofloxacin, Sulfamethoxazole, Ceftriaxone and Piperacillin with the amount of 16.67%.

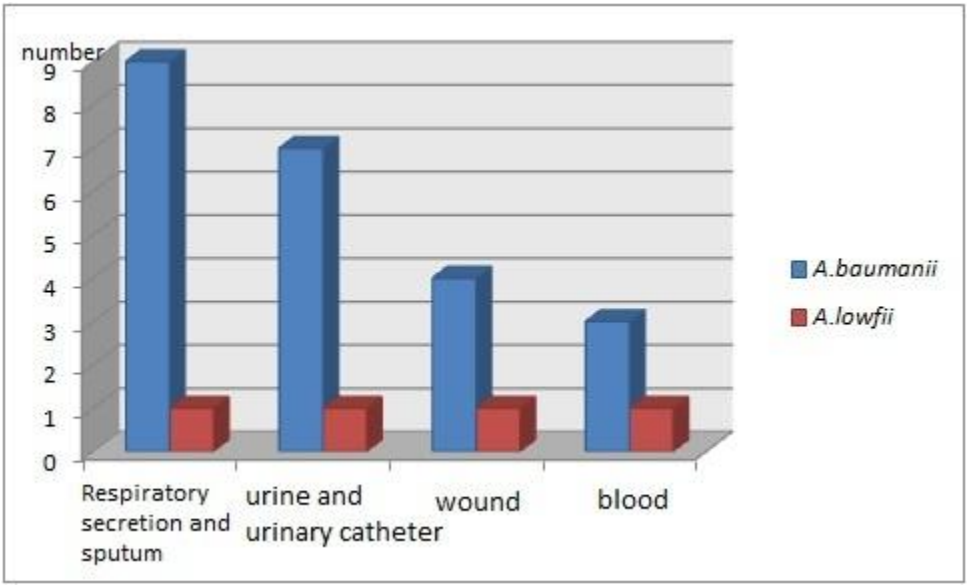

Figure 1: Frequency of *A.baumanni* and *A.lowfii* isolated from clinical samples of Anzali hospital (Iran) during 2013-2014

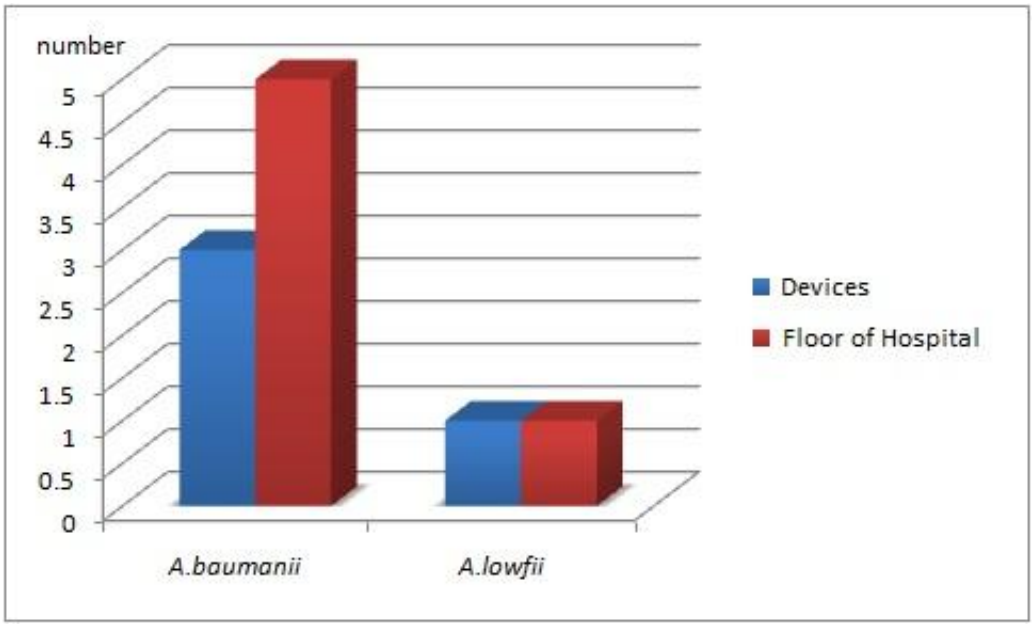

Figure 2: Frequency of *A.baumanni* and *A.lowfii* isolated from the environment of Anzali hospital (Iran) during 2013-2014

Research Article

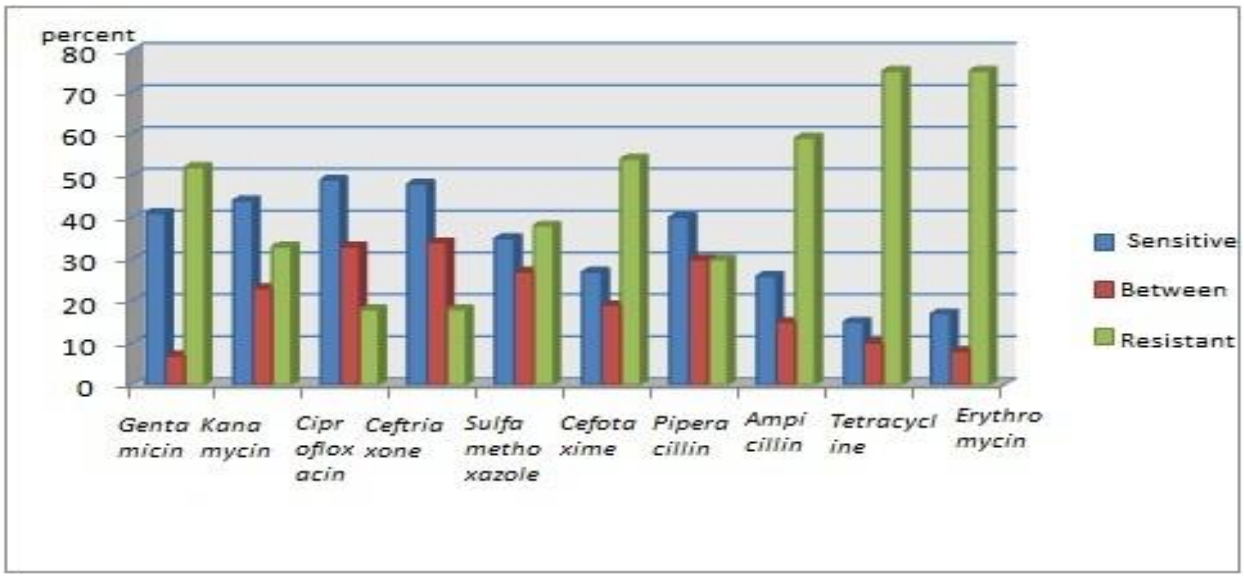

Figure 3: Antibiogram test for *A.baumanni* isolated from clinical and environmental samples of Anzali hospital (Iran) in 2013-2014

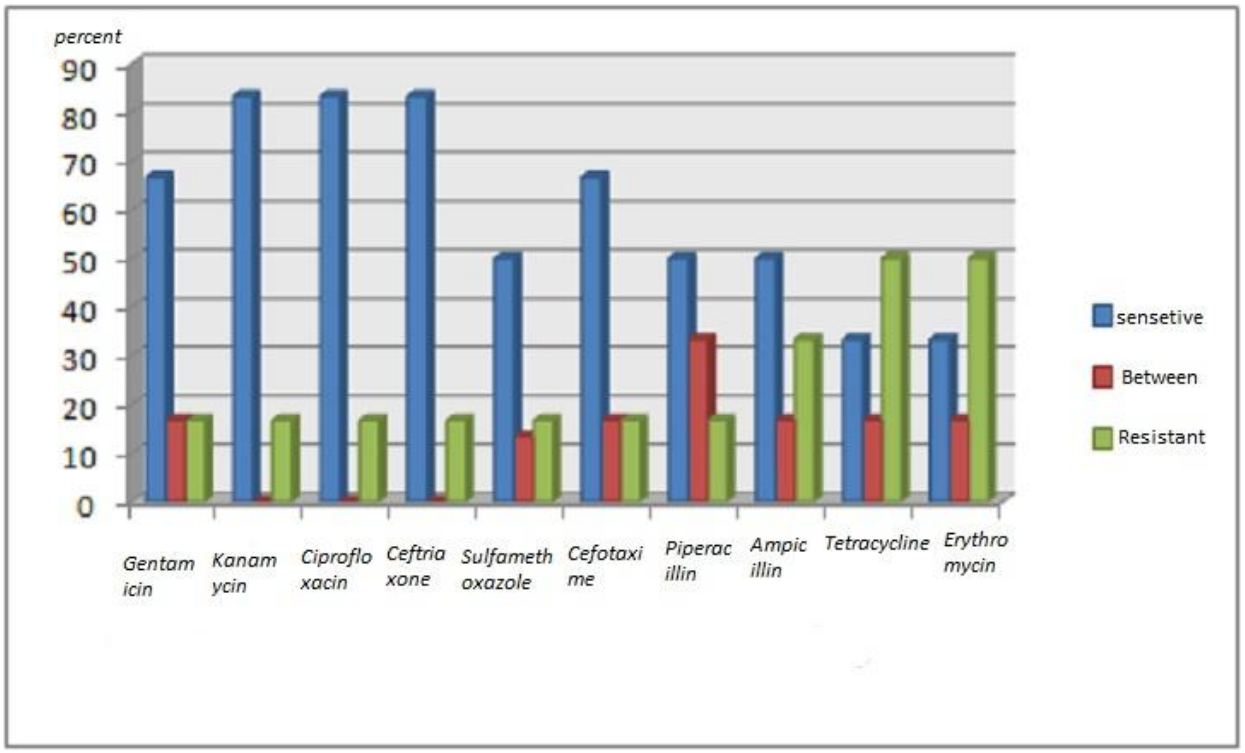

Figure 4: Antibiogram test for*A.lowfii* isolated from clinical and environmental samples of Anzali hospital (Iran) during 2013-2014

Discussion

Today, nosocomial infections have become one of the fundamental problems in the field of treatment. The cause of many nosocomial infections is gram-negative bacteria. Over the last two decades, *A.baumanni* has found an important place among the causes of nosocomial infections. Currently, *A.baumanni* is recognized as one of the important causes of nosocomial infections and major problems in

intensive care units and this is associated with the multiplicity of infections and the development of multiple resistance indices to the main groups of antibiotics (Petersen *et al.,* 2011). Findings of this study show that according to the rank average in clinical samples, *A.baumanni* and *A.lowfii* are 6.5 and 2.5, respectively.

Also, given the P-value obtained (0.014) which is lower than 0.05, there is a significant difference between clinical samples in *A.baumanni* and *A.lowfii* and there is more *A.baumanni* in clinical samples considering the rank average obtained. Furthermore, the rank average is 3.88 for hospital's environment and 2.75 for clinical samples and the P-value obtained (0.481) which is higher than 0.05 shows that there is no significant difference in *A.baumanni* between hospital setting and clinical samples. Based on information from American National Screening Systems of Nosocomial Infections, the percentage of *Acinetobacte*r pneumonia in ICU has increased from4% in 1986 to7% in 2003. In the study of Simhon *et al.,* (1990), sensitivity to imipenem decreased from 98.1% to 64.1% in 2000 and sensitivity to ciprofloxacin had a decrease from 50.5% to 13.1%. However, in our study, sensitivity to ciprofloxacin was 49% and this fact suggests an apparent difference in resistance level comparing to our study which can be related to antibiotic consumption pattern as well as environmental factors. The results of antibiotic sensitivity resistance tests in our study were obviously different from those of other studies conducted inside country by Sadeghi *et al.*,, Zandi *et al.*,, Rahbar *et al.*,, Samadi *et al.*,, YousefLangaee *et al.,* and Akbari *et al.,* because all studies indicate high antibiotic resistance to antibiotics such as Gentamicin, Kanamycin, Tetracycline, Sulfamethoxazole, Cefotaxime, Ceftriaxone, Ciprofloxacin, and Erythromycin (Zandi *et al.,* 2007; Rahbar *et al.,* 2006), but this research expresses an increase in antibiotic resistance of *A.baumanni* and *A.lowfii*.

In addition, considering the results of other studies in European countries during the years 1997 to 2008, we also find out that antibiotic resistance level (for cefotaxime, ciprofloxacin, piperacillin, tetracycline, and erythromycin) in isolates of our study is higher than others' (Asma *et al.,* 2006). Main places for *Acinetobacter* nosocomial infections have changed over time. First, urinary tract infection was prevalent in ICU and then UTIs incidence decreased which was probably associated with better watching urinary catheters and, instead, the prevalence of nosocomial pneumonia dramatically increased. In the screening program which was performed with similar instructions in the U.S., *A.baumanni* global share among respiratory tractinfectionswasapproximately9% (Wisplinghoff *et al.,* 2000).

The observed difference between studies can be due to different research methods and the resistance pattern influenced by environmental factors as well as the antimicrobial pattern used. In our research, there is less resistance for *Acinetobacter* species to carbapenems that can be owing to new use of this drug. Another reason limiting antibiotic use is the high cost of drugs. Antibiotic resistance is a global concern but it is more of a regional problem. Considering the results of recent study and other studied carried out in our country, it can be concluded that there is a high level of resistance to most frequently used antibiotic groups among *A.baumanni* isolates which shows the inefficiency of this group in the treatment of *A.baumanni* infections. Physicians should be aware of a high level of resistance to these drugs and the treatment failure resulting from using them and antibiotic consumption have to be far more limited.

ACKNOWLEDGEMENT

We are grateful to Islamic Azad University, Lhijan Branch Authorities, for their useful collaboration.

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