



Antibiogram of *Acinetobacter baumannii* isolated from Baghdad Hospitals.

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Abstract

Acinetobacter baumannii is an opportunistic bacterial pathogen primarily associated with hospital-acquired infections. It is very important to test the susceptibility of *A. baumannii* strains to antibiotics due to the property of developing multiple resistances to antimicrobial agents. The main objective of this study was to analyse the activity of 8 antibiotics against *A. baumannii*. MICs were determined by microdilution for colistin and disc diffusion method to determine MICs for 7 different antibiotics: Piperacillin, ceftriaxime, meropenem, amikacin, tetracycline, ciprofloxacin and trimethoprim-sulfamethoxazole. Results revealed that most isolates were multidrug resistant to those antibiotics. The highest percentage (94%) was Piperacillin resistant and lowest percentage (20%) was colistin resistant. Colistin is the drug of choice for treatment *A. baumannii*. However, in the last few years colistin-resistant *A. baumannii* strains have been isolated.

Keywords: *Acinetobacter baumannii*, antibiotic resistance, Baghdad hospitals, antibiogram, colistin, amikacin.

Introduction

Acinetobacter baumannii is a Gram-negative bacterium that causes nosocomial infections worldwide (Zurawski *et al.*, 2012). The outbreak of *A. baumannii* associated with United States military operations in Iraq generated special interest in this organism (Scott *et al.*, 2007). The source of *A. baumannii* infections are of exogenous in origin because of the ability of this organism to survive longer in the environment such as dry surfaces and resistant to desiccation (Weernink *et al.*, 1995; Paterson *et al.*, 2006). It can cause different infections such as respiratory tract, bloodstream, urinary tract infections, meningitis, endocarditis, and wound infections (Thom *et al.*, 2010). According to studies carried out for hospitals of Baghdad city it has been found that the highest percentage of isolation, 83.62% was of sputum specimens and the lowest percentage was from burn specimens 5.22% (Nadheema *et al.*, 2013). Another study in Hilla Teaching Hospital,

the highest percentage of isolation was obtained from urine samples (50%), the other source was wound samples (30%), burn and sputum samples (10%) (Mays, 2014). *A. baumannii* often associated with epidemic outbreaks of infections. These infections are becoming harder to treat due to the rising number of nosocomial infections having the ability to resist all antimicrobials in use including colistin (Perez *et al.*, 2007; Visca *et al.*, 2011; McConnell *et al.* 2013). Raya *et al.* (2013) noted that *A. baumannii* isolates were resistant to all representative drugs available to treatment *A. baumannii* infections in Iraq. Several studies also demonstrated in Iraq that *A. baumannii* have a high resistance to most antibiotics (Mohammed *et al.*, 2013; Al-Hamadani *et al.*, 2014; Al-Sehlawi *et al.*, 2014). The aim of this study was to investigate the recent status of antibiotic resistance of this bacterium in Baghdad city.

Materials and Methods

Bacterial strains

Fifty strains of *A. baumannii* were used in this study. Samples were collected from December 2014 to May 2015. The specimens were urine, wounds, burns, blood cultures and sputum, collected from four hospitals in Baghdad.

Isolation and identification of *Acinetobacter baumannii*

The collected specimens were cultivated directly on blood agar and MacConkey agar, incubated for 24 hrs at 37°C. The non-hemolytic opaque creamy colonies on blood agar, showed a pinkish tint colonies on MacConkey agar and grown at 44°C were chosen. All bacterial isolates were tested for Gram stain and conventional biochemical test (Forbes *et al.*, 2007);

identification results were confirmed by API 20E system.

Antibiotic susceptibility test

The susceptibility to colistin (CT) used in this study was determined for *A. baumannii* strains using microdilution method on Mueller Hinton broth, disk diffusion method on Mueller Hinton agar plates used to determine antibiotics susceptibility other 7 different antibiotics. These antibiotics were selected as they are effective against *Acinetobacter* according to Clinical Laboratories Standards: piperacillin, ceftriaxime, meropenem, amikacin, tetracyclin, ciprofloxacin and trimethoprim-sulfamethoxazole (Table 1). Using overnight culture at 0.5 McFarland standard and incubated at 37°C for 24 hrs. The results determined according to Clinical Laboratories Standards Institute (CLSI, 2011).

Table 1. Antibiotic disks used in this study

Antimicrobial	Symbol	Concentration
Piperacillin	PRL	100µg
Cefotaxime	CTX	30 µg
Meropenem	MEM	10 µg
Amikacin	AK	30 µg
Tetracyclin	TE	30 µg
Ciprofloxacin	CIP	5 µg
trimethoprim-sulfamethoxazole	TMP	10 µg

Results and Discussion

Isolation and identification of *Acinetobacter baumannii*

A. baumannii was isolated from 75 samples from wound, urine, sputum, and blood 50 isolates (66.66 %) of *A. baumannii* was identified depending on the morphological and biochemical tests, and using API 20E system. The infection rate *A. baumannii* identified samples (blood, wound, urine and sputum) was (30, 26, 12, 12) % respectively. Growth at 44°C

was positive for all *A. baumannii* isolates which showed the ability to grow at this temperature and showed a pinkish tint colonies due to non-lactose fermentation when cultured on MacConkey (Sofia *et al.*, 2004; Forbes *et al.*, 2007).

Antibiotics Susceptibility

This study showed a high level resistance of *A. baumannii* clinical isolates to most of the antibiotics under test (figure 1).

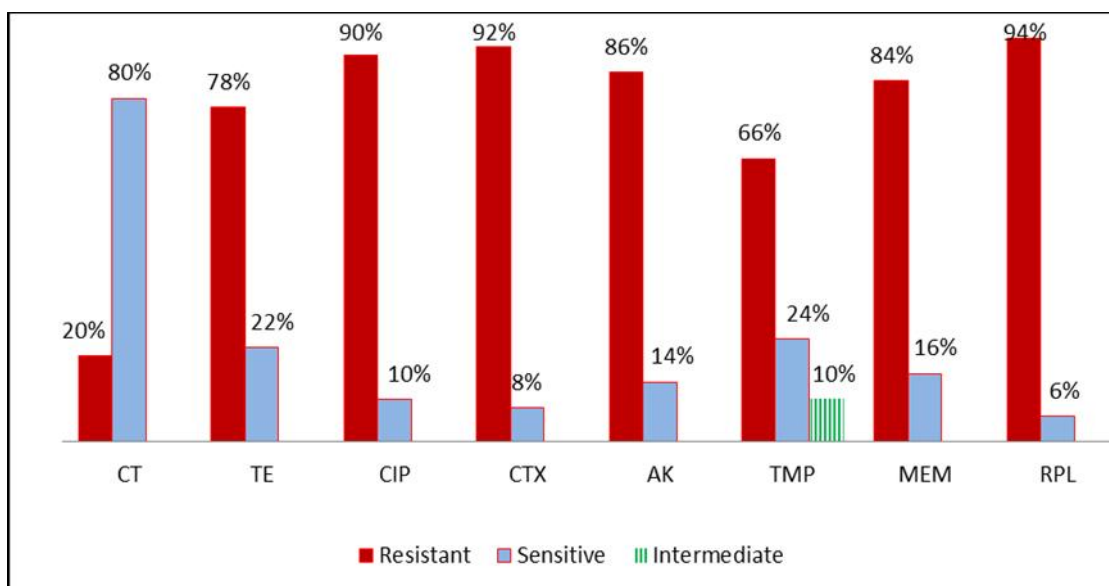


Figure 1- Antibiotic resistance, sensitive and intermediate of *A.baumannii* clinical isolates (Total isolates 50).

Similar Iraqi study in 2006 found that *A. baumannii* clinical isolates were 100% resistant to ciprofloxacin (CIP) and 82.35% resistant to tetracycline (TE) (Al-Khafaji, 2006); Study carried out in 2010 found that clinical isolates were develop 100% resistance to cefotaxime (CTX) (Al-Mash'hadani, 2010). Another study in 2013 found that 72% of *A. baumannii* clinical isolates were resistant to amikacin (AK) ,91.30% resistant to piperacillin (RPL),83.48% resistant to ciprofloxacin (CIP) and 86.09% resistant to trimethoprim-sulphamethoxazole (TMP) (Nadheema *et al.*, 2013).In study carried out by Al-Warid (2014) found that 100% of *A. baumannii* clinical isolates were resistant to piperacillin (PRL)and tetracycline

(TE), 63.63% resistant to amikacin (AK),81.81% resistance to trimethoprim-sulfamethoxazole (TMP) and only 9% resistant to polymyxin B. Al-Hamadani (2014) noted that the *Acinetobacter* spp. isolates have notably high rates of resistance to the commonly used flouroquinolones (ciprofloxacin, norfloxacin and levofloxacin). From these local studies , can be noticed that there is an increase of multidrug resistant *A. baumannii* (MDR-AB) in Iraq hospitals ,example amikacin (AK) (Figure 2),these differences in the results may be due to nexcessive use of antimicrobial agents in the hospitals in the last few years (Al-Mash'hadani , 2010; Hashim ,2011; Nadheema *et al.*, 2013).

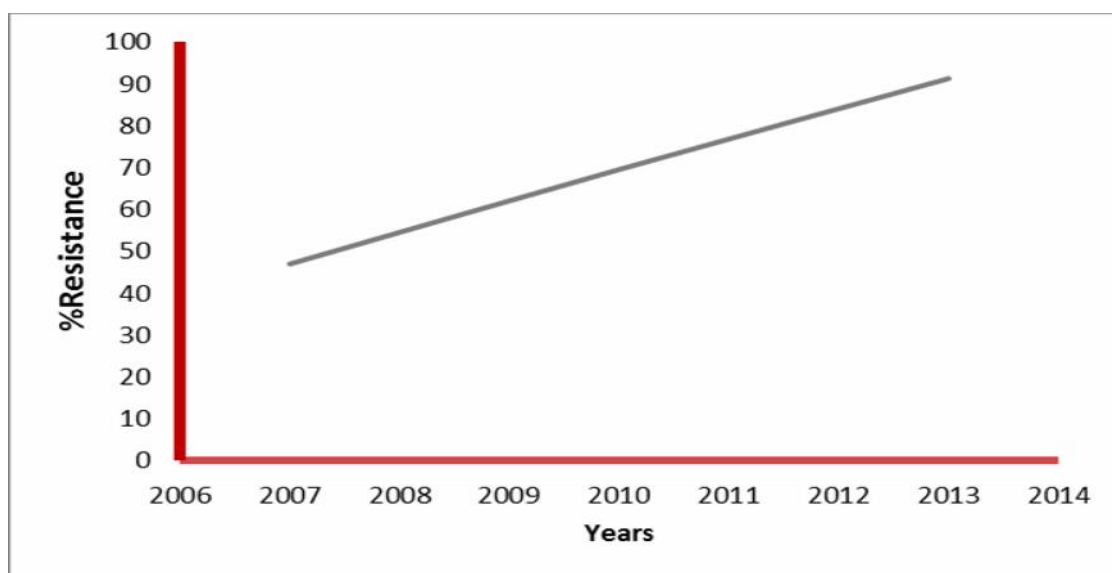


Figure 2- Increase amikacin resistance of *A.baumannii* clinical isolates in Iraq.

Although the rate of outbreaks of colistin resistant strains remains low, colistin is an antibiotic that preserve activity for a significant level (Vila and Pachon, 2012). In study on isolates obtained from samples of patients in USA Military treatment facilities who had been in Iraq found only imipenem and colistin demonstrated reliable antimicrobial activity against *A. baumannii* (Scott *et al.*, 2007); another study refer that 96% of *A. baumannii* isolated at Walter Reed Army Medical Center (WRAMC which is the destination for service members wounded in Iraq and Afghanistan) there was an increasing resistance to antibiotics (especially resistance to ceftazidime, fluoroquinolones, gentamicin, imipenem, and piperacillin/tazobactam). To treat infections with multidrug resistant *A. baumannii* (MDR-AB) the doctors increasingly resort to polymyxins, such as colistin (Kris *et al.*, 2007). In this study the results showed that 30% of colistin resistant strains were sensitive to tetracycline (TE) and trimethoprim-sulfamethoxazole (TMP), these results were in agreement with study refer that resistance to colistin can induce phenotypic changes in the outer membrane penetrability favoring other antimicrobial agents, therefore the colistin resistance isolates showed higher susceptibility rates for amikacin (AK), tetracycline (TE) and trimethoprim-sulfamethoxazole (TMP) (Rodrigo *et al.*, 2008; Laura *et al.*, 2014). They found inactivation of lipid A biosynthesis resulting in complete loss of LPS production in *A. baumannii*. (Moffatt *et al.*, 2010). The less negative charges of LPS-deficient might be the reason for a loss of affinity to colistin (Yun *et al.*, 2012).

Conclusion

A. baumannii has frequently been considered the most clinically relevant *Acinetobacter* species worldwide. It has emerged as an important nosocomial opportunistic pathogen in outbreaks of hospital infections. However, resistance to colistin has been reported all over the world, rate of outbreaks of colistin resistant strains remains low. Colistin is the most effective compared to other antibiotics in the treatment of infection *A. baumannii*.

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