The incidence of pan-drug resistance in a sample of *Acinetobacter baumannii* clinical isolates obtained from Al-Ramadi Teaching Hospital

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Abstract

Background: *Acinetobacter* infections have become more difficult to treat owing to the emergence of isolates resistant to all commonly prescribed antimicrobial drugs, particularly pan-drug resistant *Acinetobacter baumannii*.

Aim of Study: to investigate the incidence of pan-drug resistance in a sample of *A. baumannii* clinical isolates obtained from Al-Ramadi Teaching Hospital

Materials and Methods: Twenty-six *A. baumannii* were isolated from 130 wound and burn patients obtained from operative theatres and burn centre, in Al-Ramadi Teaching Hospital from October 2012 to May, 2011. Identifications were performed by standard microbiological techniques and by means of a commercial identification gallery (API 20E) and further confirmed by (API 20NE). The susceptibility of the isolates to selected major classes of anti-*A. Baumannii* agents were determined by the standard disk diffusion method as described in the guidelines of the Clinical Laboratory Standard Institute (CLSI). Appropriate tests were performed for detecting carbapenemase and metallo-β-lactamase production.

Result: Out of 26 isolates of *A. baumannii*, 6/26 (23%) were imipenem-resistant pandrug resistant (IR-PDR) *A. baumannii*, i.e. resistant to all representative drugs of available anti-*A. baumannii* in Iraq. One out of 26 (4%) was imipenem-resistant multidrug resistant (IR-MDR). All the (7) imipenem-resistant *A. baumannii* isolates were obtained from the Burn Centre, six were isolated from patients have taken meropenem in their treatment and only one patient who had not taken meropenem but admitted to the centre at the same time with other two patients suffering from Steven-Johnson syndrome have been given meropenem, all showed positive results in the metallo-β-lactamase production screening tests.

Conclusion: It is concluded that the presence of carbapenem, multidrug and pandrug resistance is evident in our sample of *A. baumannii* isolates and may be associated with the use of meropenem and inadequate infection control measures particularly in burn patients.

***Key words*:** *Acinetobacter baumannii*, Pan-drug resistance, Metallo-β-lactamase, Ramadi

Introduction

Acinetobacter spp. possesses a remarkable ability to accumulate resistance mechanisms, in particular to β-lactams (penicillin, cephalosporin and carbapenem), tetracycline, aminoglycoside and fluoroquinolones. The emergence of carbapenem resistance in this context is the most crucial issue because carbapenem are the antibiotic molecules that are used to treat patients in intensive care when all other antibiotics have failed to work [(1)]. *Acinetobacter* infections have become more difficult to treat owing to the emergence of isolates resistant to all commonly prescribed antimicrobial drugs, particularly pan-drug resistant *Acinetobacter baumannii* [(1, 2)].

More than its virulence characteristics, the main danger associated with *A. baumannii* resides in its capability to acquire antimicrobial-resistance genes extremely rapidly, leading to multidrug resistance [(4)]. This extremely rapid development of antimicrobial resistance is likely to result from the ability of *A. baumannii* to respond rapidly to challenges issued by antimicrobials, coupled with the widespread use of antimicrobials in the hospital environment. In particular, the influence of wide use of extended-spectrum cephalosporin and quinolone has been demonstrated [(3, 4)].

The potential role played by the capacity of *A. baumannii* to survive in the hospital environment in the spread of epidemic strains is reflected by the success of infection control measures, including environmental decontamination with hypochlorite solutions [(4)].

Restriction of the use of antibiotics, especially those with broad-spectrum activity and those identified as antibiotics of last resort, is a necessary complement to any infection control strategy [(5)]. The implementation of systems to monitor antimicrobial resistance and its relationship to antimicrobial use, as well as a program of antimicrobial stewardship, has been recommended. These are likely to have an impact on MDR *A. baumannii*, particularly as specific drugs favoring the emergence and dissemination of this organism are identified and restricted [(5)]. There have been many reports of *Acinetobacter baumannii* infections among American soldiers wounded in Iraq, earning it the nickname "Iraqibacter" [(6)].

The aim of this study is to investigate the incidence of pan-drug resistance in a sample of *A. baumannii* clinical isolates obtained from Al-Ramadi Teaching Hospital.

Materials and Methods

Twenty-six *A. baumannii* were isolated from 130 wound and burn patients obtained from operative theatres (urology, orthopedic and general surgery) and Burn Centre respectively, in Al-Ramadi Teaching Hospital from October to May, 2011. Identifications were performed in the hospital microbiology laboratory by staining with Gram stain, the biochemical motility, oxidase and catalase tests [(7)] and by means of a commercial identification gallery (API 20E) and further confirmed by (API 20NE). Susceptibility to selected major classes of antimicrobials including piperacillin (PRL, 100μg),

ampicillin/sulbactam (SAM, 20µg), cefotaxime (CTX, 30µg), ceftriaxone (CTR, 30µg), ceftazidime (CAZ, 30µg), cefepime (FEP, 30µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), gentamicin (GEN, 10µg), amikacin (AK, 30µg), imipenem (IMP, 10µg), colistin (CT, 10µg), and was performed using disc diffusion method [8]. All the imipenem-resistant *A. baumannii* isolates were investigated for carbapenemase production by the imipenem-EDTA double disc synergy method [9], imipenem-EDTA combined disc method [10], Hodge test [9], and the modified three dimensional methods [11], before and after the addition of EDTA as backup tests.

Results

Out of 26 isolates of *A. baumannii*, 6/26 (23%) were imipenem-resistant pandrug resistant *A. baumannii* (IR-PDR *A. baumannii*), i.e. resistant to all representative drugs PRL, CAZ, FEP, CIP, LEV, GEN, AK, IPM) of available anti-*A. Baumannii* in Iraq. All the 26 isolates were sensitive to SAM and colistin which are up to now not available In Iraq. One out of 26 (4%) was imipenem-resistant multidrug resistant *A. baumannii* (IR-MDR *A. baumannii*); this isolate was sensitive to AK, and Lev. While the other 19/26 (73%) were all imipenem-sensitive *A. baumannii* (ISAb) (Table 1). All the (7) imipenem-resistant *A. baumannii* isolates were isolated from the Burn Centre. In the screening test for carbapenemase production, our results showed that 7/26 (27%) isolates of IRAb were resistant to CTX, CTR, CAZ, FEP, and carbapenem (represented by imipenem and meropenem) and sensitive to aztreonam by disc diffusion method considering the breakpoint listed by CLSI (2011) [12]. All the seven isolates 7/7 (100%) showed positive result in the imipenem-EDTA combined disc method, Hodge test, and the modified three dimensional method before and after the addition of EDTA; while, only two out of the seven (29%) IRAb isolates showed positive results in imipenem-EDTA double disc synergy method (Figure 1 & 2, Table 2). Out of the seven IRAb isolates, six were isolated from patients taken meropenem in their treatments and only one patient who had not taken meropenem but admitted to the Burn Centre at the same time with other two patients suffering from Steven-Johnson syndrome and they have been given meropenem apparently as prophylaxis.

Table 1. Interpretive reading of *Acinetobacter baumannii* isolates susceptibility tests.

No. of Isolates	Interpretive reading											
	IPM (10µg)	PRL (100µg	CAZ (30µg)	FEP (30µg)	CTR (30µg)	CTX (30µg)	AK (30µg)	GEN (10µg)	LEV (5µg)	CIP (5µg)	SAM (20µg)	CT (10µg)
1B	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
2B	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
3B	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
4B	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**S**	**S**
5B	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**S**	**I**	**S**	**S**
6B	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
7B	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
8W	S	R	I	R	R	R	S	S	S	S	S	S
9W	S	I	S	S	R	R	S	S	S	S	S	S
10W	S	I	I	R	R	R	S	S	S	S	S	S
11W	S	S	R	R	R	R	S	S	S	S	S	S
12W	S	S	S	S	R	R	S	S	S	S	S	S
13W	S	S	S	S	R	R	S	S	S	S	S	S
14W	S	I	S	S	R	I	S	S	S	S	S	S
15W	S	S	S	S	R	I	S	S	S	S	S	S
16W	S	S	S	S	R	R	S	S	S	S	S	S
17W	S	R	S	I	R	R	S	S	S	S	S	S
18W	S	S	R	R	R	R	S	S	S	S	S	S
19W	S	R	I	I	R	R	S	S	S	S	S	S
20W	S	R	R	R	R	R	S	S	S	S	S	S
21W	S	R	I	R	R	R	S	S	S	S	S	S
22W	S	R	R	R	R	R	S	S	S	S	S	S
23W	S	S	S	S	R	R	S	S	S	S	S	S
24W	S	S	S	S	R	R	S	S	S	S	S	S
25W	S	R	R	R	R	R	S	S	S	S	S	S
26W	S	S	S	R	R	R	S	S	S	S	S	S

B: burn; W: wound. Note: all the seven isolates (100%) which were obtained from the Burn Centre were carbapenem resistant; while none (0%) of the 19 wound isolates was carbapenem resistant (i.e. this resistance was confined to burn isolates).

Table 2: Results of carbapenemase screening tests of *Acinetobacter baumannii* isolates.

No. of isolate	IMP	MEM	CTX	CTR	FEP	CAZ	ATM	DDST	CDM	Hodge test	M3DM
1B	R	R	R	R	R	R	S	+ve	+ve	+ve	+ve
2B	R	R	R	R	R	R	S	-ve	+ve	+ve	+ve
3B	R	R	R	R	R	R	S	-ve	+ve	+ve	+ve
4B	R	R	R	R	R	R	S	-ve	+ve	+ve	+ve
5B	R	R	R	R	R	R	S	-ve	+ve	+ve	+ve
6B	R	R	R	R	R	R	S	-ve	+ve	+ve	+ve
7B	R	R	R	R	R	R	S	+ve	+ve	+ve	+ve

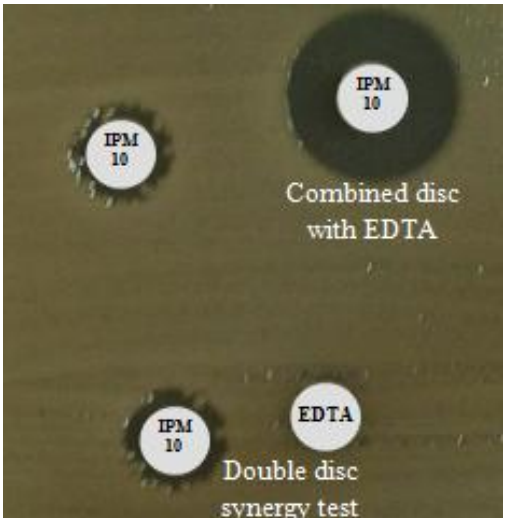

Figure 1: Performances of combined disc method and double disc synergy test using disc of IPM (10μg) and IPM (10μg) combined with EDTA (10μl of 0.1M) and disc of EDTA (10μl of 0.1M). Note: a marked synergistic effect observed with the combined disc method.

Enhanced growth (distortion) of the surface organism of local standard *E. coli* is seen near agar slits (black arrow) that contain bacterial suspensions of test *Acinetobacter baumannii* isolates, the circular well filled with 30μl of freshly prepared bacterial suspension in 0.85% saline adjusted the density to equal a McFarland 4 turbidity standard, ~10^9 CFU/ml (A, B imipenem-resistant pandrug resistant *A. baumannii*) both showed distorted inhibition zone which indicate carbapenemase production, while (C) contain imipenem sensitive *A. baumannii* isolate and (D) contain local standard *E. coli* isolate, both showed the negative results. Note: poor growth along in slit C & D and in the region lying within the inhibition zone of imipenem disc (10μg) (white arrow) reflecting susceptibility of the isolate.

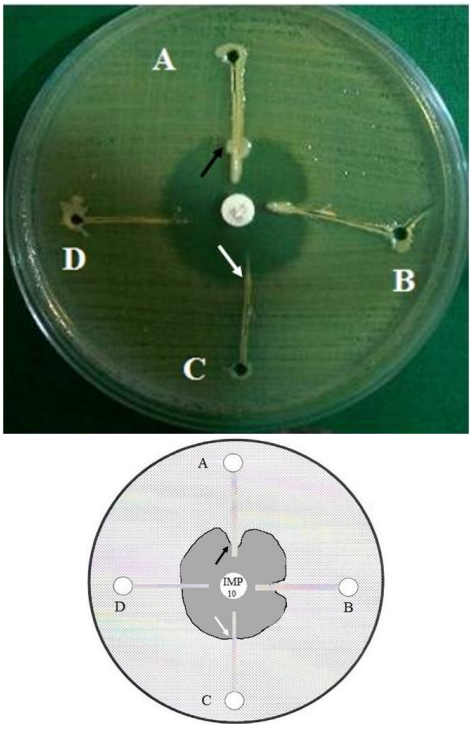

Figure 2: M3DM patterns for four isolates shown on the left, actual photograph of the Petri dish, on the right, a drawing depicting the essential details

Discussion

In the screening test for carbapenemase production, our result showed that 7/26 (27%) isolates of IRAb were resistant to CTX, CTR, CAZ, FEP, and carbapenem (represented in this study by imipenem and meropenem) and **sensitive** to aztreonam (as a pharmacological tool) and among the seven imipenem resistant *A. baumannii* isolates. All the seven isolates showed positive results with the imipenem-EDTA combined disc method [10], Hodge test [9], and the MD3M [10] as backup tests indicating that **all** the seven isolates were MBL-producing ones. All the seven isolates showed a high level of carbapenem resistance and to all other β-lactams

except **aztreonam**; this further suggests that all the seven isolates were MBL-producing [13], making them unlikely to be an oxa-type carbapenemase [14]. Further, this resistance mechanism was confined to the isolates (7/7, 100%) obtained from the Burn Centre, as it was not detected in the isolates obtained from the operating theatres (0/19, 0%). MBL–producing isolates have been increasingly reported in many geographic regions. Due to the ability of MBL-producing isolates to spread and to hydrolyze most β-lactam agents, accurate detection of this resistance phenotype by routine laboratories is essential to initiate adequate empirical therapy and to implement proper infection control practices [15].

After analyzing the initial results of this study an alarm was raised and an urgent meeting was held with the appropriate medical staff in charge for this issue in the hospital. Accordingly, appropriate aggressive infection control measures were adopted and implemented with close supervision including e.g. the institution of rigorous environmental cleaning by proper cleaning agents and disinfectants which included evaporated 7% hydrogen peroxide, and 10% sodium hypochlorite. As the disinfectant was being used previously hydrogen peroxide, purchased locally, was estimated to be of no more than 1% potency {by an expert chemist (personal communication)} and not 7% as it should have been [16]. The emergence of MBL-producing isolates suggests the laxity in the adequacy of empirical therapy, and in the appropriateness of the infection control measures. Up to our knowledge, this is the first such study on *A. baumannii* has been reported in Iraq by Iraqi scientists.

It is concluded that the presence of carbapenem, multidrug and pandrug resistance is evident in our sample of *A. baumannii* isolates and may be associated with the use of meropenem and inadequate infection control measures particularly in burn patients.

Recommendations

- Wound excision to remove nonviable tissue then adopting judicious antimicrobial therapy to decrease selection and emergence of IR-MDR *A. baumannii*.
- Judicious use of carbapenem with antibiotics stewardship programs would be the most effective measure to avoid the emergence of imipenem resistant MDR *A. baumannii* and implementation of aggressive infection control measures to ensure the eradication of IR-MDR *A. baumannii*, particularly in intensive care units.

Conflict of interests

The authors have nothing to disclose.

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