

Association Between Antibiotics And Disinfectants Resistance profiles Among *Acinetobacter Baumannii* Isolates In Zagazig University Hospitals Intensive Care Unit

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Abstract: Objectives: The study was conducted to investigate the association between the resistance profile of *Acinetobacter baumannii* isolates to antibiotics and their biocides resistance profile. In addition, to investigate the plasmid profile of multidrug resistant isolates. **Material and Methods:** Eight hundred clinical samples were obtained from medical intensive care unit of Zagazig University Hospitals including urine, sputum, pus and blood. Suspected *Acinetobacter baumannii* isolates were identified by API system. Their susceptibility to different classes of antibiotics were tested using Kirby and Bauer disk diffusion method. MICs of some antimicrobial agents and biocides that were commonly used in Zagazig University Hospitals were determined by the agar dilution method and then resistant isolates were typed by plasmid analysis method. **Results:** Eighty eight *Acinetobacter baumannii* isolates and nine cases of non-*baumannii* *Acinetobacter* were obtained from different clinical specimens. 96.6% of *Acinetobacter baumannii* isolates were multidrug resistant by Kirby and Bauer disk diffusion method. Ceftazidime MICs were the highest; and ciprofloxacin demonstrated lower MICs when tested against the clinical isolates. MICs of polymixin B and amikacin were the lowest and the isolates were more sensitive to both drugs. Also, these isolates were more susceptible to glutaraldehyde and chlorine with decreased sensitivity to ethyl alcohol. A specific plasmid profile was associated with multidrug resistance profile of *Acinetobacter baumannii* isolates. **Conclusion:** There is a wide spread of multi-drug resistant *Acinetobacter baumannii* isolates, in medical intensive care unit of the Zagazig University Hospital. Also, a significant correlation between certain antibiotic and biocides resistance was observed with specific plasmid profile of multidrug resistant isolates.

[Nissreen E. Ali, Samar S. Morsi, ElSayed A. Elgohary. **Association Between Antibiotics And Disinfectants Resistance profiles Among *Acinetobacter Baumannii* Isolates In Zagazig University Hospitals.** *Life Sci J* 2014;11(10):1-8]. (ISSN:1097-8135). <http://www.lifesciencesite.com>. 1

Keywords: *Acinetobacter baumannii*, agar dilution, multidrug resistant, biocides resistance, plasmid profile.

1. Introduction:

Acinetobacter baumannii is a non-fermentative, Gram-negative, nonmotile, oxidase-negative bacillus, which is widely distributed in many health care systems as environmental saprophytes and human colonizer.¹

Acinetobacter baumannii has emerged as a significant opportunistic pathogen which is responsible for different types of nosocomial infection in intensive care unit (ICU), such as bacteraemia, pneumonia, urinary tract infection, secondary meningitis and wound infections.²

Nosocomial infections caused by *A. baumannii* were found difficult to be managed due to emergence of multidrug resistant strains. The main contributing factor to multiple antibiotic resistance was misuse of the valuable broad spectrum antimicrobial agents within the hospitals.^{3,4}

In addition to antibiotic abuse, antiseptics and disinfectants have been used extensively in many healthcare settings for decontamination of skin, different medical devices and surfaces of hospital environment. So, a reduced susceptibility of many nosocomial pathogens, such as methicillin-resistant

Staphylococcus aureus (MRSA) and *Pseudomonas aeruginosa* to various biocides has been described.⁵ Unlike these pathogens, few studies have investigated the susceptibility to disinfectants in *Acinetobacter spp.*^{5,6} More recently, reduced susceptibility to some disinfectants by *Acinetobacter spp.* isolates has been demonstrated due to improper use of these disinfectants as repeated exposure to sub inhibitory concentrations.^{6,7,8}

Antimicrobial resistance of *Acinetobacter baumannii* isolates was determined by some genetic factors such as resistance plasmids (R-plasmids).¹ The plasmids which present in *Acinetobacter baumannii* could be readily transferred to other pathogenic bacteria by transformation and conjugation. A single resistance plasmid can carry a number of genes encoding multiple drug resistance. Thus, *Acinetobacter baumannii* is considered as an important multi-drug resistant pathogen and infection control measures must be taken to contain *Acinetobacter baumannii* infections.⁹

2. Material and methods:

Clinical samples and identification:

This study was conducted in Microbiology and Immunology department, Faculty of Medicine, Zagazig university, Egypt. The clinical cases were collected by simple random method, and different specimens, including urine (300), sputum (150), pus (250), and blood (100) were obtained from a variety of patients in the Medical Intensive Care Units (MICUs) of Zagazig University Hospitals from September 2012 to December 2013.

Presumptive diagnosis of *Acinetobacter spp.* was made by inoculation of different specimens on MacConkey agar medium and urine samples were inoculated on CLED agar (Oxoid, UK). The inoculated media were incubated at 37 °C for 24 hours. All non-lactose fermenter colonies were subjected to Gram staining, oxidase, catalase and motility tests. *Acinetobacter baumannii* isolates are presumptively diagnosed as Gram negative bacilli or cocco-bacilli, oxidase negative, non-motile, and catalase positive.⁹ Suspected isolates were confirmed by a commercial microidentification system (API 20E; bioMérieux, Marcy-L'Etoile, France).

Clinical specimens were investigated to find the frequency and distribution of *Acinetobacter baumannii* pathogens in different infections. These microorganisms were recognized as the causative agents of nosocomial infections including pneumonia, urinary tract infections, septicaemia, and wound infections if these infections appeared 48 hours after hospital admission.²

Antimicrobial susceptibility testing of *Acinetobacter baumannii* isolates

Antimicrobial susceptibility patterns of eighty eight *Acinetobacter baumannii* isolates were determined by Kirby and Bauer disk diffusion method on Mueller Hinton (MH) agar plates (Oxoid, UK). The antibiotic discs used were Ampicillin-sulbactam (10 / 10µg), Piperacillin-tazobactam (100/10µg), Trimethoprim-Sulfamethoxazol (1.25/23.75 µg), Gentamycin (10 µg), Amikacin (30 µg), Polymixin (300U), Tetracycline (30 µg), Ceftazidime (30 µg), Cefotaxim (30 µg), Imipeneme (10 µg), and Ciprofloxacin (10) µg (Oxoid, UK). The plates were then incubated at 35-37°C for 24 h. The zones of inhibition diameter were measured and interpreted as sensitive, intermediate and resistant isolates using Clinical Laboratory Standards Institute reference values, CLSI M100-S18.¹⁰

Determination of Minimal Inhibitory Concentrations (MICs) of antimicrobial agents

Minimal inhibitory concentrations of antimicrobial agents against *A. Baumannii* were determined by the agar dilution method according to the protocol recommended by the clinical Laboratory Standards Institute, CLSI, M100-S18.¹⁰ The antimicrobials selected were from different classes

that demonstrated the highest activity by Kirby Bauer disc diffusion method. They include: ceftazidime, ciprofloxacin, amikacin and polymixin B. The concentration of each antibiotic used was ranging from 1 µg/ml to 1024 µg/ml.¹⁰

Determination of Minimal Inhibitory Concentrations (MICs) of biocides

The biocides used in this study were selected according to their acceptability and frequency of use in Zagazig University Hospitals and they included sodium hypochlorite, Ethanol, and glutaraldehyde. The use-concentrations of these disinfectants in this hospital were 0.6% (6.15% diluted 10 times), 70%, and 2.5%, respectively. The susceptibility of *Acinetobacter baumannii* to these disinfectants was determined by the agar dilution method, according to the protocol recommended by CLSI M100-S14.¹¹

Serial two fold dilutions of each of the disinfectants were made; for ethyl alcohol from 100% to 3.125%. Glutaraldehyde concentration ranged from 12% to 0.32%. For Chlorine, concentration ranged from 6.25% to 0.105%. The use-concentration of each of these disinfectants and antiseptic was also included in the evaluation test.

MIC of each antibiotic and biocide was recorded as the lowest concentration that completely inhibited growth, except for a single colony or a faint haze caused by the inoculums and presented as MIC range, MIC_{50s}, and MIC_{90s}.¹¹ Standard MIC₅₀ and MIC₉₀ measurements are MICs measurement of 50% and 90% of the population, respectively.

In the present study, the DRS isolates were defined as those for which MICs of at least one among the three disinfectants were higher than the MIC₉₀ when measured by the agar dilution method as guided by CLSI M100-S14.¹¹

Plasmid analysis of *Acinetobacter baumannii*

A colony of *A. baumannii* was inoculated in 5 ml of nutrient broth and incubated overnight at 37°C in shaking water bath. Plasmid DNA was extracted using a commercial plasmid extraction kit following the instructions of the manufacture (QIAprep® Spin Miniprep kit Qiagen, Germany). Briefly, preparation and clearing of a bacterial lysate was done by centrifugation and suspension of the bacterial cells in the lysis buffer. Then, adsorption of DNA onto the QIAprep membrane and elution of plasmid DNA by adding the elution TE buffer were performed consecutively. The purified plasmid DNA was stored at -20°C.

Approximately 20 µl of the extracted plasmid and 5 µl of running dye were loaded into 1% agarose gel in TAE buffer system (Cole-Parmer, USA) with *Escherichia coli* MTCC 131 standard plasmids DNA as a molecular weight marker ; (Roche, Lewes, East Sussex).

Statistical analysis

Data of the current study was analyzed using Statistical Package for the Social Sciences (SPSS) version 15. Spearman correlation coefficient was used to detect correlation between MICs values of disinfectants and antimicrobial agents tested.

3. Results

Acinetobacter baumannii infections in the Medical Intensive Care Unit

A total of 88 (11% of the cases) *Acinetobacter baumannii* isolates were obtained from clinical specimens including blood, urine, pus, and sputum. However, non-*baumannii* *Acinetobacter* isolates constituted about 1% of the nosocomial infection pathogens. Urine was the most common source of *Acinetobacter baumannii* (53.4%) followed by sputum (18.3%). Other sources of *Acinetobacter baumannii* infections were wound infections (17%) and septicemia (11.3%). **Table 1** shows the distribution of these isolates in different samples.

Table 1. Distribution of *Acinetobacter* spp. isolates in different patient specimens.

Source\ Isolates_NO.	<i>Acinetobacter</i> spp.		Total NO. (%)
	<i>A. baumannii</i> NO. (%)	Non- <i>baumannii</i> acinetobacter NO. (%)	
Blood (100)	10 (10%)	3 (3%)	13 (13%)
Urine (300)	47 (16%)	4 (1.3%)	51 (17.3%)
Wound (250)	15 (6%)	1 (0.4%)	17 (6.4%)
Sputum (150)	16 (10.6%)	1 (0.6%)	16 (11.2%)
Total (800)	88 (11%)	9 (1%)	97 (12%)

Antimicrobial resistance patterns of *Acinetobacter baumannii* isolates

Kirby and Bauer disk diffusion tests revealed that 85 (96.6%) isolates of *Acinetobacter baumannii* were considered as multidrug resistant as they were resistant to more than three antibiotics from different

classes. *Acinetobacter baumannii* isolates that were sensitive to all antibiotic except ampicillin/sulbactam were only 3 (3.4%).

The results of antibiotic susceptibility testing by Kirby and Bauer disk diffusion method are presented in table (2).

Table 2. Antibiotics resistance patterns of *Acinetobacter baumannii* isolates

Antibiotic resistance pattern	NO. of resistant isolates	Percentage
SAM	3	3.4%
SAM, GM, SXT, CAZ, AK, CIP, PB	18	20.45%
SAM, PIP\TAZ, IPM, GM, CTX, CAZ	20	22.72%
SAM, PIP\TAZ, IPM, GM, CTX, SXT, TC	43	48.86%
SAM, PIP\TAZ, IPM, GM, CTX, CAZ, TC, SXT, CIP, AK	4	4.54%

SAM: Ampicillin-sulbactam, PIP/TAZ: Piperacillin-tazobactam, CTX: Cefotaxime, CAZ: Ceftazidime, GM: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin, IPM: Imipenem, SXT: Trimethoprim-sulfamethoxazole, PB: Polymixin B, TC: Tetracyclin.

Minimal inhibitory concentrations of antimicrobials tested against clinical isolates of *Acinetobacter baumannii*

Polymixin B demonstrated the lowest MICs among the antimicrobial agents tested with least level of resistance. This followed by amikacin MICs. The

determined MICs of ceftazidime was the highest followed by MICs of ciprofloxacin. MIC₅₀ and MIC₉₀ for the tested antibiotics were presented in table 3.

The distribution of *A. baumannii* isolates within different ranges of MICs of the tested antimicrobials were presented in **figure 1**.

Table 3. Minimal inhibitory concentrations of antibiotics tested against *Acinetobacter baumannii* isolates by agar dilution susceptibility testing.

Antibiotic	MIC Range (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
Ceftazidime	32-1024	256	512
Ciprofloxacin	1-256	32	256
Amikacin	4-64	16	64
Polymixin	1-32	2	16

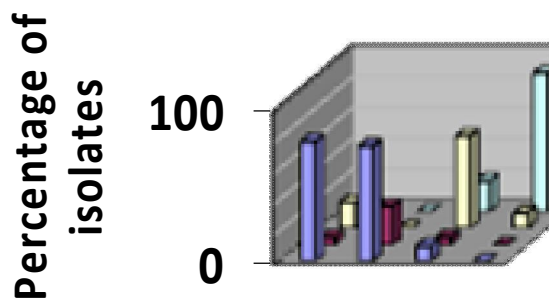


Figure 1. The distribution of *Acinetobacter baumannii* isolates among different MICs antibiotics tested by agar dilution susceptibility test. Pol: Polymixin, Amk: Amikacin, Cip: Ciprofloxacin, Cef: Ceftazidime

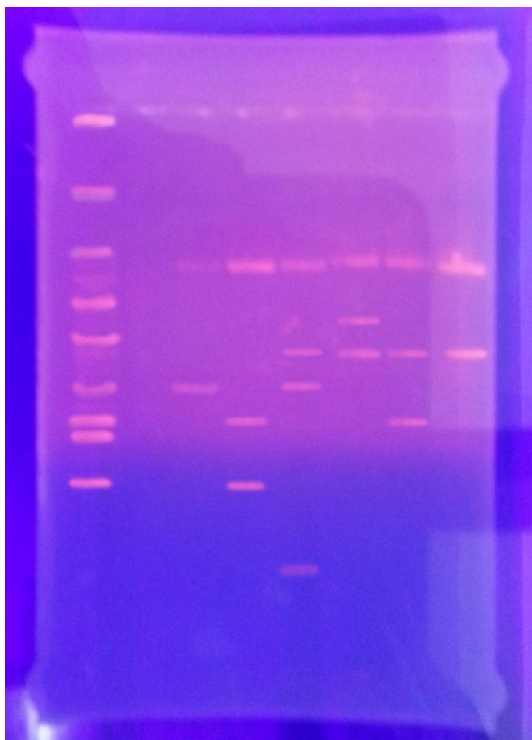


Figure 2. Plasmid profile of *A. baumannii* clinical isolates. Lane 1, *E.coli* (pRK2013); MTCC harboured eight different plasmids with known molecular weight 54.8, 4.8, 3.7, 3.4, 2.6, 2.0, 1.8, 1.4. Lanes from 3 to 8 represent isolates with R-plasmids > 54kbp. Lane 2 was for plasmid free isolates.

Minimal inhibitory concentrations of biocides tested against clinical isolates of *Acinetobacter baumannii*

Acinetobacter baumannii tended to be more susceptible to gluteraldehyde and chlorine. However, MICs of ethyl alcohol was relatively higher than

those of the other disinfectants as presented in table (4). As a result, 15 (17%) *A. Baumannii* isolates were defined as DRS isolates.

Correlation between MICs values of biocides and antimicrobial agents tested against *Acinetobacter baumannii* isolates

MICs of the chlorine and gluteraldehyde for eighty eight clinical isolates were significantly correlated with those of amikacin and polymixin. Also, chlorine MICs were correlated with those of ciprofloxacin; ($P < 0.05$). However, there was no significant correlation between MICs values of the three disinfectants with those of Ceftazidime ($P > 0.05$) as shown in table (5).

Table 4. MICs of Biocides tested against *Acinetobacter baumannii* isolates by agar dilution susceptibility testing

Biocides agents	MIC Range	MIC ₅₀	MIC ₉₀
Ethyl alcohol	50-100%	70%	>100%
Chlorine	0.105-3%	0.3%	0.75%
Gluteraldehyde	0.75-12%	1.5%	2%

Table 5. Correlation between MICs of biocides and antimicrobial agents against *Acinetobacter baumannii* isolates

Antimicrobial agents	Spearman correlation coefficient (P value)		
	Ethyl alcohol r (P)	Chlorine r (P)	Gluteraldehyde r (P)
Ceftazidime	0.26 (0.27)	0.10 (0.66)	0.14 (0.55)
Ciprofloxacin	0.35 (0.13)	0.47 (0.03)*	0.39 (0.08)
Amikacin	0.22 (0.34)	0.82 (0.000)**	0.56 (0.002)**
Polymixin	0.26 (0.36)	0.75 (0.000)**	0.58 (0.001)**

* P -value < 0.05 Significant; ** P -value < 0.001; highly significant

Plasmid profile of multidrug resistant clinical isolates of *Acinetobacter baumannii*

The analysis of plasmid in multidrug resistant and sensitive isolates of *Acinetobacter baumannii* revealed the presence of multiple plasmids from 1 to 3 with molecular weight ranging from 1 to 7.30 kbp. These Plasmids were found in 80 of 88 isolates (90.9%), and no plasmids were found in 8 isolates (9.1%).

Representative plasmid of specific molecular weight above 54 Kbp was only found in multidrug resistant (85 isolates) including 17 disinfectants resistant strain (DRS) of *Acinetobacter baumannii* and absent in the sensitive isolates (Figure 2).

4. Discussion :

Nosocomial infections caused by multidrug resistant Gram-negative bacilli has proved to be a significant problem.¹² A characteristic feature of these infections is increased the importance of strictly aerobic Gram-negative bacilli including *Pseudomonas aeruginosa*, *Stenotrophomonas (Xanthomonas) maltophilia*, and *Acinetobacter spp.*¹³

Acinetobacter baumannii has been considered as a major cause of significant opportunistic infections such as bacteraemia, pneumonia, urinary tract infection, secondary meningitis, burn and wound infections.¹⁴

In the present study, *Acinetobacter baumannii* was responsible for 11% of nosocomial infections in intensive care unit (ICU) patients. This result is consistent with that obtained by the work of Joshi². Also, Patwardhan and his colleagues⁹ reported that *Acinetobacter baumannii* responsible for about 10 per cent of nosocomial infections in ICU patients. However, Mindolli and his colleagues¹⁵ found that *Acinetobacter baumannii* isolates accounted for 27% of the infections reported in ICU.

In association with Patwardhan and his colleagues⁹, *Acinetobacter baumannii* isolated from UTI in the present study was much higher than that reported by Prashanth and Badrinath.¹⁶ However the study done by Patwardhan and his colleagues⁹, revealed that the urinary tract infection accounted for 44.4% of *Acinetobacter baumannii* infections. In association with the result obtained by Sadeghifarda and his colleagues¹², it was demonstrated that 23.2% of *Acinetobacter baumannii* isolated from ICU patients responsible for septicemia. Therefore, these bacteria can enter and persist into the blood stream and cause life-threatening multiple-drug resistant septicemia and disseminated infections.

Multidrug-resistant (MDR) *A. baumannii* is a rapidly emerging problem in healthcare settings as it limits the therapeutic options in infected patients.¹⁴ In the present study and in association with the studies done by Patwardhan and his colleagues⁹; Prashanth and Badrinath¹⁶, multidrug resistant *Acinetobacter baumannii* is responsible for majority of infections as more than 95 per cent isolates of *Acinetobacter baumannii* were found resistant to 11 antibiotics by disc diffusion method.

This study revealed high percentage of multidrug resistance with different patterns of resistance. However, Nowroozi and his colleagues¹⁷ demonstrated that all *Acinetobacter baumannii* cases (100%) had negative inhibition zones for Ciprofloxacin, Amikacin, co-Trimoxazole, Ceftriaxone and Ceftazidime and most cases (86.1%) for tetracycline and Gentamicin. Also, Sadeghifarda and his colleagues¹² found that all *Acinetobacter*

baumannii strains isolated were had 100 % resistance to at least 11 used antibiotics. This is similar to the study conducted by Capoor and his colleagues¹⁸. This presented huge ability for *Acinetobacter baumannii* to acquire resistance genes and cause serious infection in humans. The difference in the sensitivity pattern was due to environmental factors and different pattern of antimicrobial use.

A. baumannii capability to replicate in the hospital environment is facilitated both by tolerance to adverse conditions and multidrug resistance, the latter also favoring endemic infections through antibiotic-selective pressure.¹⁹ This gives an urgent alarm for the hazardous and quick development of *A. baumannii* resistance against most effective antibiotics, which in turn needs close monitoring and precise typing techniques as these highly resistant strains might spread to other regions of the whole world in a short time.

Antibiotics from different groups were tested against *A. baumannii* isolates by agar dilution method and revealed that majority of isolates tolerated more than 256 µg/ml of ceftazidime and ciprofloxacin antibiotics. In this study, the isolates percentage that was resistant to ceftazidime were higher than that obtained by Patwardhan and his colleagues⁹ who found that only about 80% of isolates were resistant to ceftazidime. Our results regarding aminoglycosides and quinolones resistance were against those reported by Patwardhan and his colleagues⁹ who revealed that quinolones resistance was less as compared to aminoglycosides. These differences could be due to different environmental distributions and various control policies.

This multidrug resistance mostly determines the pathogenicity of *Acinetobacter baumannii* because it has no specific growth requirements and it is able to grow at different temperatures and pH conditions, thereby contributing to transmission.¹² Also, the intrinsic resistance to many antimicrobial agents, contributes to the organism persistence and enables it to spread in the hospital setting.^{20,21}

Control of nosocomial infections caused by *A. baumannii* should include effective disinfectants as well as appropriate antibiotics.²² Generally, Gram-negative bacteria are generally less susceptible to antibiotic and biocides than Gram-positive species. Multiple disinfectants with various mode of action are used in hospitals, including glutaraldehyde, formaldehyde and chloride-releasing agents compounds. These agents are recommended for patients and health care items.²³ Also, alcohol is an important antiseptic agents commonly used in hospitals.²⁴

In this study and in agreement with the results obtained by Patwardhan and his colleagues⁹,

Acinetobacter baumannii isolates that were tested for their sensitivity to the biocides that are routinely used in zagazig university hospitals. Including, chlorine, gluteraldehyde, and alcohol tended to be susceptible to gluteraldehyde and chlorine as the MIC₉₀s of chlorine and gluteraldehyde were approximately half of their in-use concentrations. Also, Kawamura-Sato and his colleagues⁸ found that MIC₉₀s of the tested disinfectants were lower than their in-use concentrations. However, The previous reports by Martró and his colleagues²⁵ and Wisplinghoff and his colleagues²⁶, demonstrated no apparent development of resistance to disinfectants among clinically isolated *Acinetobacter* spp. In the current study, MICs of ethyl alcohol were relatively higher than those obtained by Cardoso and his colleagues who found that 70% ethyl alcohol removed significantly more bacteria from heavily contaminated hands than other decontaminating agents.²⁷

In agreement with Sadeghifarda and his colleagues¹², the DRS (17%) of *A. baumannii* isolates tended to demonstrate reduced susceptibility to two or more disinfectants. This percentage is higher than that obtained by Kawamura-Sato and his colleagues who revealed that DRS *A. baumannii* constituted about 10% of the isolates.⁸

Susceptibility to disinfectants must be carefully checked regularly if several multidrug-resistant *A. baumannii* are frequently isolated from health care systems despite proper control measures.²²

Most of the DRS *Acinetobacter baumannii* isolated in this study tended to demonstrate reduced susceptibility to some antimicrobial agents. MICs of the chlorine and gluteraldehyde were significantly correlated with those of amikacin and polymixin B. Also chlorine MICs were correlated with those of ciprofloxacin; ($P < 0.05$). However, there was no significant correlation between MICs of the three disinfectants with those of Ceftazidime ($P > 0.05$). Thus far, the positive correlation between bacterial antibiotic resistance and disinfectant resistance has been suggested. In association with our results, Russell and his colleagues²⁸ revealed that chlorhexidine gluconate resistance in *Pseudomonas stutzeri* correlated with resistance to polymyxin B, gentamicin, erythromycin and ampicillin. Also, Dance²⁹, reported that a hospital outbreak caused by a strain of *Proteus mirabilis* demonstrated resistance to several antimicrobial agents, including gentamicin as well as disinfectants as chlorhexidine gluconate. In addition, Johnson and his colleagues³⁰ found that MRSA isolates in tertiary care adult and pediatric populations were resistant to both CHG and QAC.

Thus, the increased isolation of *Acinetobacter* spp. that had acquired multiple resistance to antimicrobials would be a good indicator for early

recognition of the emergence of *Acinetobacter baumannii* DRS isolates in healthcare settings.

These findings may well suggest difference in the mode of acquisition of resistance to disinfectants and the development of multiple antimicrobial resistance in *Acinetobacter baumannii* isolated in different regions whole over the world.⁹

In *Acinetobacter*-associated nosocomial infection, the major problem encountered by ICU clinicians relates to the readily transferable antimicrobial resistance expressed by this organism.³¹ This transferable resistance is carried on R-plasmids. The clinical *A. baumannii* isolate as well as unrelated environmental *A. baumannii* isolate had a similar antibiotic resistance plasmid suggesting spread of this genetic character.³²

This study and in association with the studies done by Sadeghifarda and his colleagues¹² and Paredesi and his colleagues³³; revealed that resistance of *A. baumannii* to both antibiotics and disinfectants was significantly associated with a plasmid of a specific molecular weight above 54 kbp. This provided evidence on the feasibility of using more precise molecular typing and detection methods for tracing *A. baumannii* resistance against antibiotics and disinfectants. On the other hand, multiple antibiotic resistant *Acinetobacter baumannii*, was isolated by Joshi and his colleagues previously but plasmid borne nature of antibiotic resistance has been reported only in a few cases in India.²

4. Conclusions

Nosocomial infections caused by *Acinetobacter baumannii* is considered as emerging problem in medical ICU of Zagazig University Hospitals. Most of isolates were actually multi-drug resistant. Also, multidrug resistant *Acinetobacter baumannii* isolates demonstrated disinfectant resistance to commonly used disinfectants in the hospitals. In addition, this study highlighted high precision of plasmid profile in predicting antibiotic resistance. These results encourage us to evaluate new treatment and decontamination options.

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5/29/2014