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STUDY THE VIRULENCE FACTORS AND PATTERNS OF ANTIBIOTICS RESISTANCE IN *ACINETOBACTER BAUMANNII* ISOLATED FROM HOSPITALIZED PATIENTS IN BAGHDAD CITY

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ABSTRACT

Acinetobacter baumannii is an opportunistic nosocomiala multidrug resistance(MDR) pathogen, so arising largely infections by this bacteria specially in immuno-compromised patients and ability to survive in hospital environments and it became important human pathogen so , virulence factor and antibiotic resistance are playing important role in infections but few studies in Iraq about this bacteria there for present study aimed to study the virulence factors among A*cinetobacter baumannii* isolated from Hospitalized patients among hospitals in Baghdad city and evaluate the antibiotics resistance in A*cinetobacter baumannii* isolate.

Thirty-nine isolate of A*cinetobacter baumannii* were isolated during period March to October 2015 from various clinical source from laboratories of bacteriology in different hospitals of Baghdad city then diagnosis and identification by classical methods and vitek 2system, and study virulence factors as form Biofilm; Capsule formation; Pellicle assay; hemolysin production and various enzymes so evaluated the antimicrobial resistance for twelve different antibiotics.

Acinetobacter baumannii was more isolated from Wound and Burn swab(38.5%) so (28.2%) isolated from both Urine and sputum , whilst (5.1%) form blood, and high percentage of *Acinetobacter baumannii* (43.5%) in age group(40-60) years, whilst only (2.5%) in age less than 20 years, as well as high percentage (59.4%) founded in males, Also biochemical test were positive for catalase and citrate, while negative for each of Oxidase, indole , Urease , Lactose fermentation , motility and hemolysin , also all isolate were positive for gelatinase and 21 isolate positive for Protease whilst 29 isolate positive for both Lipase and Capsule as well as 18 isolate positive for Lecithinase and 38, 33 isolate positive for heamolysin production and Pellicle assay respectively . so 32 of A*cinetobacter baumannii* isolates were positive for biofilm formation, also current study appearance all A*cinetobacter baumannii* isolates were found resistant to ampicillin, Cefoxitin and tetracycline (100%), whilst low resistance to Imipenem and Piperacillin 58.9, 15.4% respectively.

High percentage of A*cinetobacter baumannii isolated* from burn swab, whilst low percentage from blood so high percentage isolated from age group (40-60) years and frommales patients more isolate compared to females .as well as Acinetobacter *baumannii* isolates have multiple virulence factors that apparent all A*cinetobacter baumannii* isolates have gelatinase activity whilst varied result other factors, and highest resistance of isolates to Ampicillin, Cefoxitin and Tetracycline.

Keyword: *Acinetobacter baumannii,* virulence factors, antimicrobial resistance, multidrug resistance (MDR).

INTRODUCTION

Acinetobacter baumannii is a multidrug resistance (MDR) and an opportunistic nosocomial pathogen has many features as obligate aerobic, gram negative, coccobacillus, nonmotile, oxidase negative, catalase-positive [Peleg *et al.,* 2008], first isolated of these bacteria, by using minimal media enriched with calcium acetate [Beijerinck, 2008] and described as Micrococcus calcoaceticus, so it has many virulence factors [Young, 2007].

Incidence of these bacteria increased proportion among immunocompromised individuals [McConnell *et al.,* 2013] and hospital-acquired infections but the rates infections has increased in the summer [McDonald et al., 1999] and high mortality rate of community-acquired infection because it has capacity to prosper and survive for prolonged periods on environmental surfaces of hospital [Urban et al., 2003], via its interact with different types of surfaces as abiotic surface in hospital as Cell phones, medical equipment , linen and furniture [Borer *et al*., 2005], especially in intensive care units (ICUs) as well as difficult treatment infections of *Acinetobacter baumannii* [Van Looveren, 2004], and production of pili (fimbriae), toxins, enzymes, as well as iron chelators that important contribute in success of bacterial infection [deBreij *et al.,* 2010]. So, interact with biotic surface as human tissue and obtain the essential nutrients from these tissue as iron so ability to damage host tissues by producing gela-

tinases and proteinases [Tomaras, 2008]. Spread of Acinetobacter baumannii from hospital to other hospital by rotation of medical staff , patients and students *[*Landman, 2000*], therefore can be attributed as* a hospital pathogen [Oncül, 2002], recently the World Health Organization (WHO) has identified as one of the most important problems to health of human [Bassetti *et al.*, 2011]. many pathogenic bacteria as MDR pathogens named (ESKAPE) which meaning *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.* [Rice, 2008]. So, these bacteria named Iraqi bacter because it high incidences of these bacteria in blood (bacteremia) among US Army [CDC, 2004].

MATERIALS AND METHODS

Isolation and Identification: During period from March to October 2015 were isolated 39 isolate of Acinetobacter baumannii, from different hospitals in Baghdad city isolate from various clinical source (blood sample; urine; sputum, as well as burn and wound burn swab) then diagnostic and identification by classical methods and vitek 2 system (bioMerieux, France).

Identification of some virulence factors: Study virulence factors as Biofilm; Capsule and various enzymes as Gelatinase, Protease, Lipase, Pellicle assay and Lecithinase as well as hemolysin production.

1- **Biofilm formation (by Microtiter plates):** Adhesion of bacteria to 96-well microtiter plate surfaces was carried out by inoculating 20µL of overnight grown culture in Luria–bertoni broth containing 180µL of the growth medium. Four were left un-inoculated as negative controls. Then plates were incubated at 37 °C for 72 h and staining by crystal violet 1% w//v and then quantified at 570nm after solubilization with ethanol–acetone (Merritt *etal.*,2005), experiments were carried out in triplicates, the degree of biofilm was calculated as the equation: Biofilm degree = Mean OD_{570} of tested bacteria –Mean OD_{570} of control

2- Gelatinase test: Inoculated the colonies in Luria agar and incubated overnight at37°C, then cooled at 4°C for five hours, positive result by appearance turbid halo (Sechi *et al.*, 2004).

3- Protease and Lipase production: Streaking on the surface of skim milk and egg-yolk agar plates and incubated at37ºCfor 24 hr. The clear zone was adjacent the streaked, that indicates protease and Lipase production [Collee *et al*., 1996].

4- Lecithinase: using Baird-Parker medium. The formation of an opaque halo indicated a positive result [Matos*etal.*,1995].

5- Hemolysin production: streaking on Columbia agar plates and incubated at 30ºC for48h. a clear zone indicated positive results.

6-Pellicle assay: from each isolate inoculated in 5mlof MH broth tubes and incubated at 25°C for 5 days, positive results as white layer on the surface of MH broth (Marti*etal*.,2011).

Antibiotics resistance test: by using Vitek2 resistance test system for twelve different antibiotics including: Amikacin, Amoxicillin, Ampicillin, Cefeime, Cefotaxim, Cefoxitin, Ciprofloxacin, Gentamicin, Imipenem, Nitrofurantion, Norfloxacin, Piperacillin which were obtained from bioMerieux-France.

Statistical analysis: Analyses of all data were done by SPSS Package program. Frequencies and percentage of the parameters were done, and categorical data were compared using Chi- squared.

RESULTS AND DISCUSSION

Table 1: Number and percentage of *acinetobacter baumannii* according to the Clinical source of isolate

Virulence Factor	Results		Total
	Positive	Negative	
Gelatinase	**39**	**0**	39
Heamolysin	**38**	1	
Biofilm	**32**	**7**	
Protease	**21**	18	
Lipase	**29**	**10**	
Capsule	**29**	**10**	
Lecitienase	**18**	**21**	
Pellicle assay	**33**	**6**	

Table 1 show the results obtained from bacterial culture and Vitek system that showed A*cinetobacter baumannii* was more isolate (38.5%) isolated from Wound and Burn swab so (28.2%) isolated from both Urine specimen and sputum whilst only (5.1%) form blood sample, because contaminated the environmental of hospital and health care were transmitted the bacteria and playing important role in this outbreak of *Acinetobacter baumannii.*

These findings are in agreement with AL-Warid & AL-Thahab (2014) in their study reported high percentage of A*cinetobacter baumannii isolated* from burn swab (6.25%) whilst low percentage from blood (0.93%) [AL-Warid & AL-Thahab, 2014], whilst Japoni *et al.*, (2011) showed *Acinetobacter* spp were mostly isolated from the blood (39.8%).

Table 2: Biochemical tests & its results for *Acinetobacter baumannii*

Biochemical test	Result
Catalase	+
Citrate utilization	+
Hemolysin production	-
Indole production	-
Lactose fermentation	-
Motility	-
Oxidase	-
Urease production	-
Kliglar iron agar	Alkaline slant / bottom No change / No gas/ No H_2S.

As in table 2, the biochemical tests results given by *Acinetobacter baumunnii* were positive results for all catalase and citrate, while negative for each of Oxidase, Indole production, Urease production, Lactose fermentation. Motility and Hemolysin production, as well as Kliglar iron agar test that gave Alkaline slant / bottom no change / no gas/ -H2S.

This supports the findings of several other authors in similar studies as Sofia, 2004, who reported A*cinetobacter baumunnii* catalase positive results whilst non-motile and negative results for oxidase [Sofia *et al.,* 2004].

Table 3: Virulencefactor for A*cinetobacter baumannii*

Clinical source	Number (39)	Percentage (%)
Wound and Burn swab	15	38.5
Urine specimen	11	28.2
Sputum	11	28.2
Blood	2	5.1
Total	39	100

Table 3 showed all isolate have positive results for gelatinase and 21 isolate positive results for Protease whilst 29 isolate positive results for both Lipase and capsule, but 18 isolate has positive results for Lecithin's as well as 38, 33 isolate positive results for Hemolysin production and Pellicle assay respectively. This finding is consistent with data obtained by Abdulla *et al.,* (2015), which showed fifteen isolates were positive to gelatinase activity whilst varied result in pellicle formation [Abdulla et al., 2015].

As recent report by AL-Warid and AL-Thahab (2014) showed that all isolate shave the ability to produce biofilm, gelatinase, and pellicle formation (100%) so 36, 54, 54% for Lecithinase, Capsule andLipase, while 72% for Protease.

In this study 32of 39 *Acinetobacter baumannii* isolates were positive for biofilm formation, these results which supports the findings of other studies that indicate that 16 of 20strains of Acinetobacter positive results for biofilm (Sechietal, 2004) and 64 of 86*A. baumannii* isolates were positive for biofilm formation as strong, medium and weak forming biofilms as 10, 27, 27% respectively [Cevahir et al., 2008].

Table 4: Resistance of A*cinetobacter baumannii* to Antibiotics.

Antibiotic	Resistance		Sensitive	
	No.	%	No.	%
Amikacin	**25**	64.2	14	35.8
Amoxicillin	**31**	79.5	8	20.5
Ampicillin	**39**	100	0	0
Cefeime	**37**	94.9	2	5.1
Cefotaxim	**34**	87.2	5	12.8
Cefoxitin	**39**	100	0	0
Ciprofloxacin	**35**	89.7	4	10.3
Gentamicin	**31**	79.5	8	20.5
Imipenem	**23**	58.9	16	41.1
Nitrofurantion	**37**	94.8	2	5.2
Norfloxacin	**37**	94.8	2	5.2
Piperacillin	**6**	15.4	33	84.6
Tetracycline	**39**	100	0	0

All A*cinetobacter baumannii* isolates were found resistant to Ampicillin, Cefoxitin and Tetracycline (100%) as well as (94.9, 94.8, 94.8)% respectively for Cefeime, Nitrofurantion and Norfloxacin whilst low resistance to Imipenem and Piperacillin as (58.9, 15.4)% respectively as mentioned in (Table 4).

In Iraq at laboratory of Babylon University, showed highest resistance of *Acinetobacter baumannii* to cefotaxime (93%), amikacin (80%), ciprofloxacin (80%), tetracycline (60%) and imipenem (53%) [Abdulla *etal.*,2015], soJaponi*etal.*2011 showed 77.3, 63.6, 61.4% of *Acinetobacter* isolates were susceptible to imipenem, tobramycin, ampicillin respectively while 26.1, 25, 23.8, 20.4, 19.3, 18.2% respectively to ciprofloxacin, amikacin, norfloxacin, gentamicin, cefepime and ceftazidime) respectively.

During the last decade, increased infections of A*cinetobacter baumannii* isolates that resistant to almost all antibiotics [Goossens, 2005], because

Acinetobacter baumannii strains haves ability to form biofilm on biotic and a biotic surface [Cai *et al*., 2012] so it have multiple bacterial virulence factors that play important role in pathogenesis o f *Acinetobacter baumannii* infections, as well as high resistance of A*cinetobacter* to antibiotics and limited using alternative effective antibiotics. More likely, acquired the resistance genes by genetic elements as plasmids, integrons and transposons (Perez *et al*., 2007), as well as most important virulence factors (biofilm) that correlated with resistance of *Acinetobacter* to antibiotics.

Conclusion:

1- High percentage of *Acinetobacter baumannii isolated* from burn swab, whilst low percentage from blood.
2- High percentage of A*cinetobacter baumannii* isolated from age group 40-60 years, whilst low percentage from age less than 20 years and from male patients more isolate this bacterium compared to females.
3- *Acinetobacter baumannii* isolates have multiple virulence factors that play important role in infections that apparent all *Acinetobacter baumannii* isolates have gelatinase activity whilst varied result in Protease, Lipase, Lecithinase, Capsule, hemolysin production, biofilm and Pellicle assay.
4- Highest resistance of *Acinetobacter baumannii* isolates to Ampicillin, Cefoxitin and Tetracycline whilst low resistance to Imipenem and Piperacillin.

Recommendation:

1- In the future study recommended investigation the role of patients, medical staff and environmental of hospital in Contaminated and transmitted the bacterial infection in hospital.
2- Isolating the genes that responsible for multidrug resistance (MDR) and study how can silence these genes.

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