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Research Article

COLONIZATION OF MULTI-DRUG RESISTANT (MDR) *ACINETOBACTER BAUMANNII* ISOLATED FROM TERTIARY HOSPITALS IN EGYPT AND THE POSSIBLE ROLE OF THE OUTER MEMBRANE PROTEIN (OMPA)

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ABSTRACT

This study aimed to investigate the virulence (adhesion and invasion) of clinically isolated multidrug resistant (MDR) *A. baumannii* on different mammalian cell lines. It also determined the role of the outer membrane protein (OmpA) extracted from such isolates in cytotoxicity to these cell lines. In this study, hundred thirty- seven clinical isolates were obtained from intensive care units (ICUs) at tertiary hospitals in Cairo, Egypt and were identified by API system. Identification was confirmed by polymerase chain reaction (PCR) amplification of the *oxa-51* gene. Based on their susceptibility to different antimicrobial agents, tested by disc diffusion method, isolates were classified into different groups. Representative isolates of each group were assayed for adherence and invasion on HEp-2, A549 and Hela cells. OmpA was then extracted from selected strains. The cytotoxicity and caspase activation of the extracted OmpA was determined on different cell lines. The isolates were found to harbor the *oxa-51* gene. Multidrug resistance was accompanied by higher binding capabilities on HEp-2 and A549 cells than Hela cells. A relation between cytotoxicity and Caspase activation was suspected among the isolates. These results suggested that apoptosis induced by OmpA of MDR *A. baumannii* might be attributed to caspase activation, though other mechanism could not be ruled out. In conclusion, *A. baumannii* has the ability to cause adhesion and invasion on respiratory cell other than other types of cells. OmpA has a vital role in the pathogenesis as it causes apoptosis to the respiratory cells.

Keywords: Adhesion, Invasion, *A. baumannii,* OmpA, cytotoxicity, caspase

INTRODUCTION

Acinetobacter baumannii is a Gram- negative opportunistic nosocomial pathogen of increasing concerns to health care system. It was identified as one of the most common pathogens in ICUs all over the world[1]. Infection by *A. baumannii* causes high incidence of morbidity and mortality among hospitalized patients[2]. In addition to its intrinsic resistance to several antimicrobial agents, *A. baumannii* has an endless ability to acquire antimicrobial resistance probably due to harboring several genomic islands containing multiple resistance genes. Several factors have been associated with virulence of *A. baumannii*. Of which, Outer membrane protein A of *Acinetobacter baumannii* (AbOmpA) is responsible for adherence and invasion of host cells impairing its function and inducing programmed eukaryotic cell death (apoptosis)[3]. It also inhibits complement-mediated bacterial lysis. Purified OmpA gets localized to the mitochondria and leads to the release of proapoptotic molecules such as cytochrome *c*, apoptosis-inducing factor (AIF) and caspases activation[4]. Caspases are present as inactive proenzymes that are activated by proteolytic cleavage. Caspases 9 and 3 are situated at critical junctions in apoptosis pathways[5].

MATERIALS AND METHODS

Bacterial Strains

A total of 137 non-duplicated clinical isolates were recovered from clinical specimens of hospitalized patients admitted to the ICUs in Kasr Al Ainy hospital and the National Cancer Institute, Cairo, Egypt. Isolates were cultured on MacConkey agar and were maintained at -75°C in Luria-Bertani (LB) containing 30 % (v/v) glycerol.

Approval of the study protocol was received from the Ethical Review Board of each of Cairo University, October University for Modern Sciences and Arts (M1/EC1/2014PHD). This study is carried out as per International conference of Harmonization-Good Clinical Practices Guidelines (ICH-GCP).

Bacterial Identification

Bacterial isolates were identified by API 20E strips (bioMerieux, Marcy l'Etoile, France) according to manufacturer's protocol for *Enterobacteriaceae* and non-enteric bacteria. The identification was confirmed by polymerase chain reaction (PCR) amplification of the *oxa-51* gene consistently found in *A. baumannii*. PCR amplification was done using the primers; F-5'-TAA TGC TTT GAT CGG CCT TG-3' and R-5'-TGG ATT GCA CTT CAT CTT GG-3', as described previously[6].

Antimicrobial Susceptibility Testing

All isolates were tested for susceptibility to different classes of antimicrobial agents using the disk-diffusion method, as described by the clinical and laboratory standards institute (CLSI)[7]. Commercial discs (Oxoid, UK) of the following antibiotics (µg/disc) were used: amoxicillin (AM,10), amoxicillin-clavulanic acid (AMC,20/10), amikacin (AK,30), gentamicin (CN,10), ofloxacin (OF,5), aztreonam (ATM,30), cefotaxime (CTX,30), meropenem (MEM,10), imipenem (IMP,10), trimethoprim-sulphamethoxazole (SXT,1.25/23.75) and doxycycline (DO,30).

Maintenance of Cell Lines

Human bronchial epithelial cells (A549), Human laryngeal carcinoma cells (HEp-2), and cervical cancer cells (Hela cells) were used in adherence, invasion and cytotoxicity assays. All cells were supplied from the holding company for biological products and vaccines (VACSERA, Giza, Egypt). The cell lines were cultivated in plastic bottles containing RPMI media (Sigma-Aldrich, Gillingham, UK) for A549 and Hela cells while HEp-2 cells were cultivated in MEM-Earle's media (Sigma–Aldrich, Gillingham, UK). After supplementing with 10% fetal bovine serum (FBS), the cell lines were incubated at 37°C in an atmosphere containing 5 % CO_2 for 48 h. Monolayers were subsequently observed under inverted microscope.

Adherence Assay

Adherence assay was performed as described by Choi et al. 2008. Briefly, all cell lines were seeded at a density of 10^5 per ml in 24 well-tissue culture plates (Nunc, Denmark). Bacterial cells for adherence assay were cultured in LB broth at 37°C for 24 h, harvested and the inoculum size for assay was adjusted to 10^6 colony forming unit (CFU)/ml. Aliquots of 500 µl of bacterial suspensions were added to 85-90% confluent monolayer of the cell lines. The same amount of cells treated with phosphate buffer saline (PBS) were used as a control for normalization[8]. The infected cell lines were incubated in Co_2 incubator for 1 hour to allow adhesion of bacteria. Afterwards, the cells were washed with trypsin. Aliquots of 500 µl were cultured on nutrient agar for colony counting. Adherence of the tested strains was calculated as the average of the total number of colonies forming units (CFU) based on the dilutions performed during plating. Colonies were counted on the plates and calculate the number of CFU of adhered bacteria and of the inoculum by averaging each series of dilutions. Only plates with between 10-300 colonies should be counted.

Invasion Assay

Cell lines and bacterial inoculum were prepared as described above by Choi et al.2008. Infected cell lines were incubated for 12 h. afterwards, cell lines were washed three times with PBS and fresh culture medium containing 300 µg/ml of gentamicin (CN) was added to eliminate all extracellular bacteria. Incubation was resumed for 1 h. The cells were then washed with PBS and lysed with 0.1% Triton X-100 for 20 min. at 37°C as described previously[8]. Bacterial colonies were enumerated by plating 500 µl of the culture on nutrient agar for colony counting after incubation. Control experiments were also included.

Detection of the Outer Membrane Protein Gene

Presence of the gene encoding OmpA was verified by PCR. For this purpose, specific primers: F-5'-CAG CAG CAA CAG AGA CTA CG-3', R- 5'-TCT TGT CAC CCA CCC ATT CT-3'were designed for amplification of *ompA* gene based on the wild-type gene sequence of the standard strains; *A. baumannii* ATCC19606 (accession number: AY485227) published in the GenBank nucleotide sequence database (www.ncbi.nlm.nih.gov). Genomic DNA was extracted using Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Amplification was performed in a final volume of 25 µL containing 1 µL of each primer at a concentration of 10 µM, 12.5 µl MyTaq™ Red Mix, and approximately 100 ng of chromosomal DNA. PCR reactions were performed using T Advanced thermal cycler (Biometra, Germany) with an initial denaturing cycle at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, annealing temperature for 30 s, and 72°C for 1 min, with a final extension step at 72°C for 5 min.

Extraction of the Outer Membrane Protein

Outer membrane protein A was extracted from the selected MDR isolates as previously described [9]. Bacteria were grown in LB broth (250 ml) at 37°C for 24 h. After 24 h. culture, bacteria were collected by centrifugation. The pellet was suspended in 50mM Tris buffer, pH7.5. Bacterial cells were sonicated using ultrasonic processor under cold conditions for 1 minute. The suspension containing the cell envelope was treated with DNase (10 µg/ml, for 15 min. at room temperature) and was subjected to ultracentrifugation for 30 min. at 100000 g. After ultracentrifugation, total membrane fraction was obtained as pellet. 2% Sodium lauroyl sarcosinate was added to the pellet for 30 min. at room temperature which solubilized the inner membrane. Further ultra-centrifugation was applied to the sample for 30min. at 100000 g and outer membrane was obtained as pellet. The outer membranes were loaded on the sephacryl gel separating column with elution buffer consisting of 0.1% SDS, 0.1 mM EDTA, 0.2 M $LiCl_2$, 50 mM Tris-HCl pH 7.5[4]. The extracted protein concentration was determined by the biuret method using the biuret reagent (Thermo Fischer Scientific, USA).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

The identity of OmpA extracted from different isolates was confirmed by the molecular weight determined by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done for analyzing proteins based on differences in their molecular size with a standard molecular weight used as size marker. Approximately 25 µg proteins (samples of the isolated outer membrane protein) were preheated then loaded on the wells. The electrophoresis was run at 120V for 1 h. After electrophoresis, the gel was stained with Coomassie blue R-250[10].

Determination of OmpA Cytotoxicity

The cells were prepared as previously described by Choi et al.2008. The cells were allowed to adhere overnight, and the culture medium was replaced with fresh assay medium supplemented with 2% FBS. The cells were treated with various concentrations of the outer membrane protein (serial dilution starting from200 µg/ml) for up to 72 h. *A. baumannii* cytotoxicity was initially assessed quantitatively by monitoring the mitochondrial reduction activity using the MTT dye[11]. The colorimetric reaction was started by adding of 20µl of MTT dye and incubated for 30 min. The absorbance of colorimetric assay was read using ELISA reader at a wavelength of 570 nm. The effective concentration, IC_{50}, is defined as the concentration of the purified protein which causes about 50% cell death. IC_{50} was measured by using masterplex reader fit program.

Measurement of Cell Death

Cells were seeded as described previously by Choi et al.2008. After 24 h., the medium was replaced with fresh assay medium supplemented with 2% fetal bovine serum containing an IC_{50} dose of the protein. In order to determine induction of apoptosis, cells were harvested at 24 h. post treatment. The cells were washed with phosphate buffered saline (PBS) and were collected. They were stained with FITC-conjugated Annexin V and propidium iodide (PI) (BD Pharmingen, USA) as described by Lee et al.2006[12]. The samples were then analyzed via flow cytometry according to manufacturer protocol.

Determination of Caspase Activation

Cells were prepared as previously described by Choi et al.2008. The cells were treated with an effective concentration of protein (IC_{50}) for 6 h. After treatment, the culture medium was discarded, and the cells were washed with PBS and gently cell culture medium was removed from the wells. Appropriate volume of cell lysis buffer was added to each culture well (MaxDiscoverycaspase-3 colorimetric detection kit) [4]. The plate was shake for 10 min. to facilitate cell lysis .100µl of Caspase-3 reaction buffer were added to the cell lysates according to manufacturer protocol (MaxDiscoverycaspase-3 colorimetric detection kit). The absorbance was measured immediately using ELISA reader (initial reaction). After 30 min., the absorbance was measured again (final reaction). The difference between the final and the initial results were calculated. The results were compared with the control result according to manufacturer instructions.

Statistical Analysis

Data of adherence and invasion are expressed as the means ± standard deviation (SD) of the indicated number of experiments. Statistical significance was assessed via Student's *t* tests with *P* values <0.05 considered to be statistically significant.

Compliance with Ethical Standards
Ethics Approval and Consent to Participate

Approval of the study protocol was received from the Ethical Review Board of each of Cairo University, October University for Modern Sciences and Arts (M1/EC1/2014PHD)

RESULTS

***A. baumannii* Identification**

A total of 137 isolates were identified as *A. baumannii* using conventional phenotypic detection methods with subsequent confirmation with API system. The results were confirmed by detection for *oxa-51* gene which is unique to these species as shown in Fig 1. Most of the isolates were recovered mainly from sputum (n=122, 89%), followed by wound specimen (n=8, 6%) then blood (n=6, 4%) and urine (n=1,1%).

Antimicrobial Susceptibility Patterns and Antibiogram-based Phenotyping of *A. baumannii* Isolates

The antimicrobial resistance patterns showed higher rates of resistance among *A. baumannii* isolates. All *A. baumannii* isolates were (100%) resistant to amoxicillin, amoxicillin-calvulanic acid, cefotaxime, in addition to 97%, 91% of the isolates were resistant to aztreonam and ofloxacin respectively. The resistant rates for carbapenem antibiotics including imipenem and meropenem were 88.5 % and 88%, respectively.

Moreover, the resistance rate was detected as 84% for trimethoprim-sulphamethoxazole, 70% for gentamicin, 71% for amikacin as well as 70% of isolates revealed resistance to doxycycline as shown in Fig 2. According to the resistance profile, isolates were classified in to 17 classes as shown in Table 1, designated as A1 to A17, depending upon their resistance to the antimicrobial agents tested.

Adhesion and Invasion Assay

To evaluate the involvement of OmpA in *A. baumannii* adherence and invasion of A549 cells and HEp-2 cells, we studied the adherence and invasion of MDR *A. baumannii* on different cells. The ability *A. baumannii* isolates to adhere to abiotic surfaces has been observed in different studies.[13, 14]

In the cell adherence assay, the total number of adherent bacteria to HEp-2 cells ranged between67300to11000 bacteria/ plate whiles the adherent bacteria in case of A549 ranged between 61000 to13800 bacteria/ plate. However, in comparison with Hela cells, the number of adherent cells ranged between 14000 to1230 bacteria/ plate as shown in Fig 4.

In the cell invasion assay, the results reached up to180000 bacteria in A459 cells, 94000 bacteria in HEp-2 cells while it reached up to 8200 only in Hela cells as shown in Fig 4.

Detection of OmpA Gene

The presence of OmpA in the isolates was screened by the conventional PCR and it was detected at 337bp.OmpA is one of the most conserved protein in *A. baumannii*

Characterization of the *A. baumannii* OmpA Protein Using SDS-PAGE

SDS-PAGE analysis of the purified OmpA protein (expected size, 38.8 kDa) appeared as a broad band with an apparent molecular mass of around 40 kDa as shown in Fig 5.

Determination the Cytotoxicity of OmpA on the Different Cell Lines

OmpA is considered a possible important virulence factor in *A. baumannii*. Selected isolates gave different concentration of IC_{50} starting from5.69µg/ml reaching to more than 200µg/ml as shown in Table (2). In addition, The IC_{50} value obtained for OmpA with the A549 cells was approximately slightly higher than the IC_{50} in HEp-2 cells. HEp-2 cells and A549 cells were treated with IC_{50} and the results of caspase-3 were higher especially in the lower IC_{50} values. Based on morphological criteria, cell death associated with several signs as the cells began to round up, and a large number of them became swollen, usually detaching from the plate surface as shown inHEp-2 cells in Fig 6.

Determination of the Apoptosis by Flow Cytometry

To determine the possible interaction of AbOmpA with epithelial cells, the binding of AbOmpA to various types of epithelial cells was analyzed by using the flow cytometry through detecting the number of dead cells which can be represented by quadrant C4(early apoptosis), quadrantC3(represented live cells). HEp-2 cells showed 66.3% dead cells and A549 cells showed 47% dead cells. However, in the control, there was only 3% of dead cells as shown in Fig 7.

Table 1: Antibiotypes of *A. baumannii* based on the Antimicrobial resistance pattern

Antibiotypes	Antimicrobial resistance pattern	Number of isolates (%*)
A1	Resistant to all antimicrobial classes used	58(42%)
A2	Resistant to all antimicrobial classes except CN,AK	10 (7%)
A3	Resistant to all antimicrobial classes except DO	13 (10%)
A4	Resistant to all antimicrobial classes except DO,CN	5(4%)
A5	Resistant to all antimicrobial classes except DO,OF	4 (3%)
A6	Resistant to all antimicrobial classes except CN,SXT, DO	5(4%)
A7	Resistant to all antimicrobial classes except CN, AK,SXT,OF	3 (2%)
A8	Resistant to all antimicrobial classes except ATM, IPM,MEM	3 (2%)
A9	Resistant to all antimicrobial classes except MEM,IPM	3 (2%)
A10	Resistant to all antimicrobial classes except CN,AK,IPM,MEM,DO	2 (1%)
A11	Resistant to all antimicrobial classes except CN,OF,DO	2 (1%)
A12	Resistant to all antimicrobial classes except CN,AK,SXT,DO,OF	2 (1%)
A13	Resistant to all antimicrobial classes except AK,CN,IPM,MEM,OF	5 (4%)
A14	Resistant to all antimicrobial classes except CN,AK,IPM,MEM,SXT,OF	3 (2%)
A15	Resistant to AM,AMC,CTX,DO	3 (2%)
A16	Resistant to AM, AMC, ATM,SXT	3 (2%)
A17	Resistant to all antimicrobial classes except CN	13 (10%)

*Percentages correlated to the total number of isolates. AM, amoxicillin; CN, gentamicin; AK, amikacin; DO, doxycycline; ATM, aztreonam; IMP, imipenem; MEM, meropenem; OF, ofloxacin; SXT, trimethoprim-sulphamethoxazole; CTX, cefotaxime; AMC, amoxicillin-clavulanic acid.

Table 2: Determination the cytotoxicity and caspase of OmpA on A549 cells& HEp-2 cells

Sample	A549 Cells		HEp-2 Cells	
	IC50µg/ml	**Caspase**	**IC50 µg/ml**	**Caspase**
A1	18	0.697	17.8	0.693
A2	200	0.341	76.83	0.574
A3	8.8	0.982	5.616	1.23
A4	96.9	0.4	52.06	0.428
A5	>200	-	>200	-
A6	>200	-	>200	-
A7	54.3	0.584	8.472	0.97
A8	99	0.482	28.945	0.43

Eight isolates were randomly selected from the antibiogram table that mainly exhibited high resistance profile (from A1 to A8). caspase activation result wasn't detected for the isolate that has IC50 >200 µg/ml.

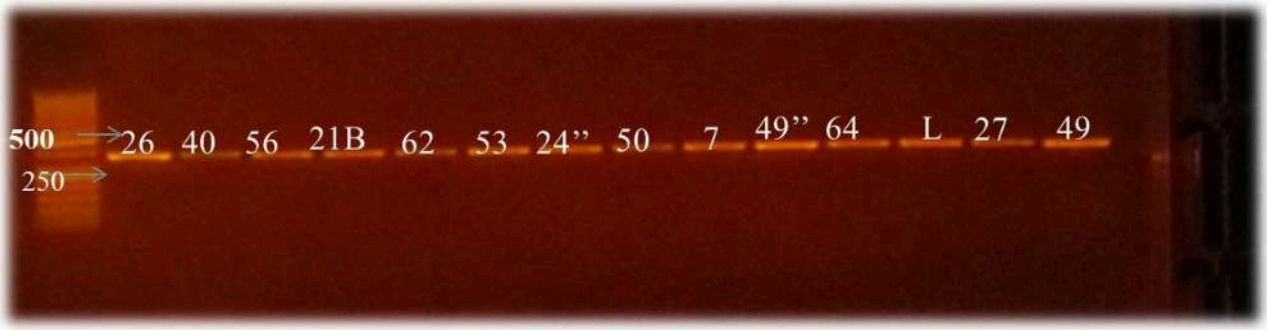

Figure 1: Detection of oxa-51 gene among the isolates, using gene ruler 50bp.
Random isolates were selected from the different antibiotypes (A1-A17) in the antimicrobial resistance pattern.

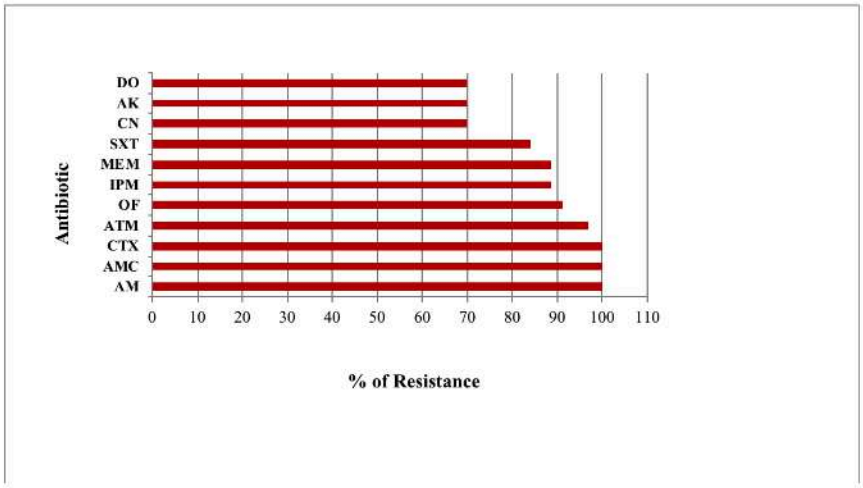

Figure 2: Antibiotic sensitivity profile of *A. baumannii* isolates

In vitro antibiotic susceptibilities of the clinical isolates of *A. baumannii* determined using the disk-diffusion method. AM, amoxicillin; AMC, Amoxicillin-clavulanic acid; CN, gentamicin; AK, amikacin; DO, doxycycline; ATM, aztreonam; IMP, imipenem; MEM, meropenem; OF, ofloxacin; SXT, trimethoprim-sulphamethoxazole; CTX, cefotaxime; % of Resistance: represents the percentage of the resistant isolates.

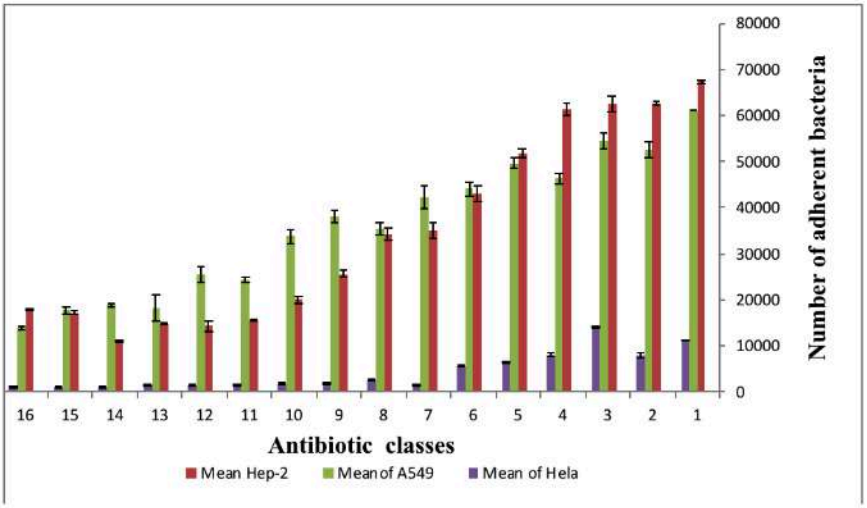

Figure 3: Detecting the Adhesion of *A. baumannii* on (HEp-2, A549, Hela) cell line

HEp-2, A549, Hela were infected with representative *A. baumannii* isolates from different antibiotypes (A1-A16) for 1 h. Results represent the mean and standard deviation of three separate experiments on separately days.

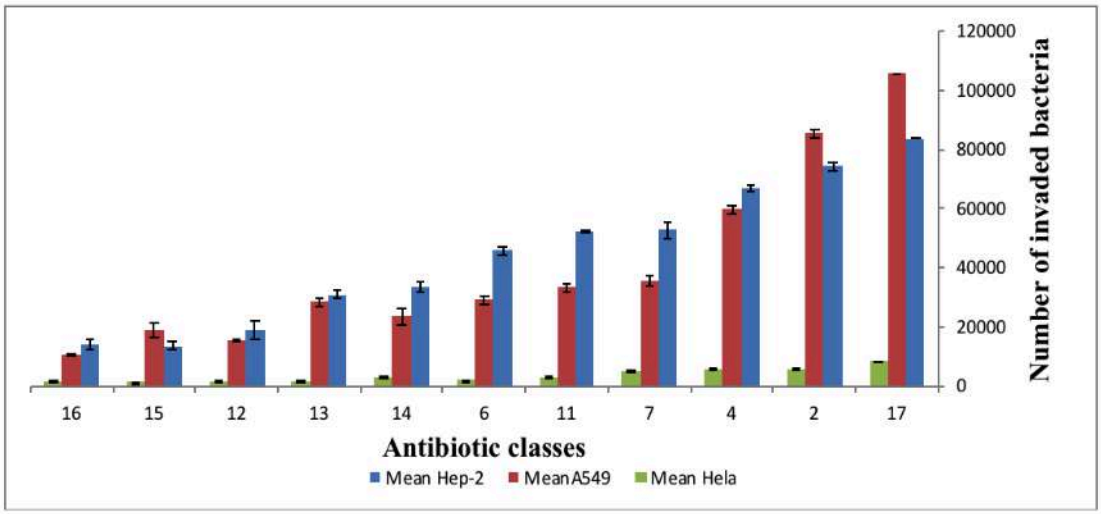

Figure 4: Detecting the invasion of *A. baumannii* on (HEp-2, A549, Hela) cell line

HEp-2, A549, Hela were infected with representative *A. baumannii* isolates from different classes. Results represent the mean and standard deviation of three separate experiments on separately days. Isolates selected in invasion assay should be sensitive to CN.

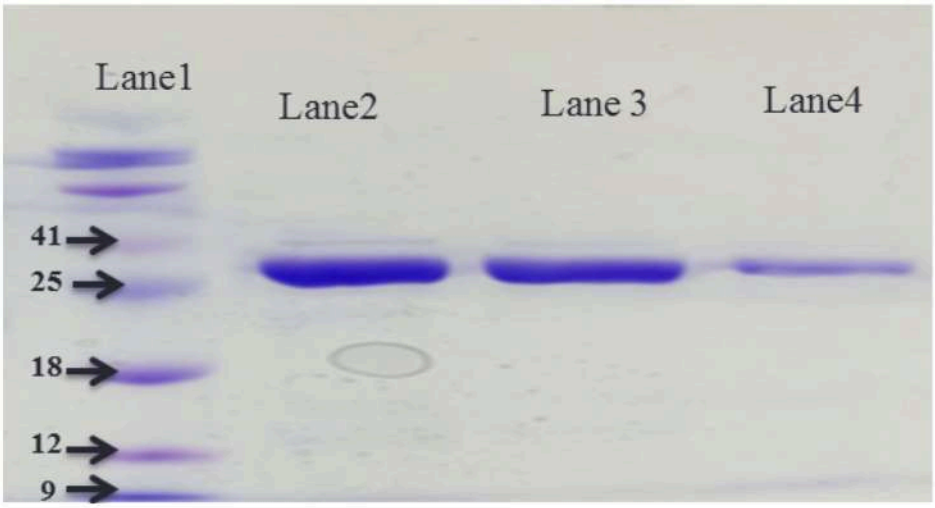

Figure 5: SDS-PAGE analysis of the purified OmpA of *A. baumannii*
lane1: represent the standard protein marker while lane 2 ,3, 4 are samples of the purified OmpA containing 5 g of the purified protein The gels were analyzed by Coomassie blue staining. The bands containing the standard proteins are indicated by arrowheads.

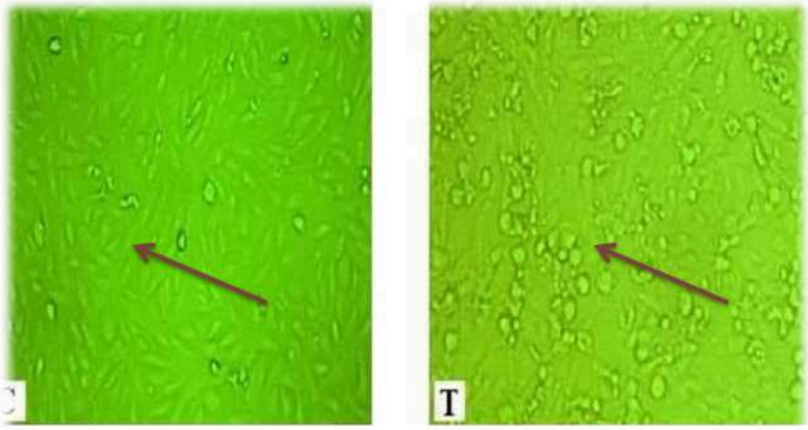

Figure 6: Morphological changes for HEp-2 cells; C: control without Infection, T: cells infected with OmpA of *A. baumannii*.
Cell retraction and shrinkage suggested apoptosis. The morphological changes and damage observed support the cell viability results which suggest lack of metabolic activity linked to the inhibition of cell proliferation thus cell damage and cell death features.

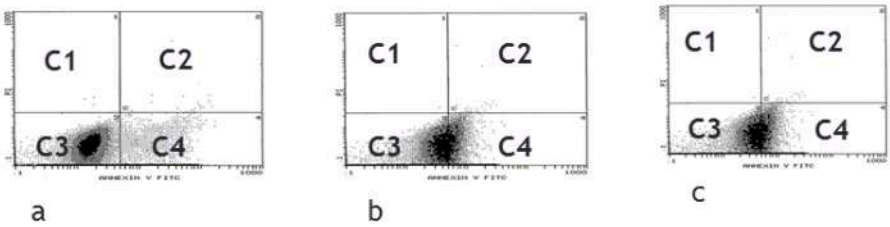

Figure 7: Flow cytometric analysis of cell death induced by the OmpA from *A. baumannii*.
a: HEp-2control which has C3(live cells) > C4(dead cells), b: HEp-2 cell infected with OmpA which has C4 > C3,
c: A549 cells infected with OmpA where C4 >C3

DISCUSSION

A. baumannii is one of the human pathogens with the highest prevalence around the world. Among 137 isolates from infected patients in ICUs included in the current study, a considerably most of the isolates were recovered mainly from sputum (89%).A survey in U.S. hospitals in2010 showed that the majority of the isolates (57.6%) were from the respiratory tract.[15] *A. baumannii* isolates in this study, contained the *oxa*-51 gene and this result was in agreement with other studies.[16, 17]

Compared with other Gram negative bacteria, *A. baumannii* develops resistance rapidly, due to its intrinsic characteristics and inappropriate use of antibiotics.[18]. Carbapenems were the most commonly implicated antibiotics used in *A. baumannii* infections, however in our results, carbapenem drugs including imipenem and meropenem, showed higher resistance frequencies of 88.5 % and 88%, respectively.

A. baumannii is known to cause infection in a variety of different tissues, colonization of the host occurs with varying frequency at different sites of the body. This variation may be the result of differing capacity of *A. baumannii* to adhere to and invade host

cells present at those body sites. In the current study, it is noteworthy that adhesion and invasion of the isolates were higher in lung derived cells (A549&HEp-2 cells), which is regarded as a primary site of *A. baumannii* colonization, than any other cells (Hela cells) (1.8×10^5&9.4×10^4 CFU VS.8.2×10^3CFU,*P*-value< 0.05 in different isolates).These results were in consistent with Choi et al who mentioned that the binding capacity of *A. baumannii* in respiratory tract-derived epithelial cells was higher than that of non-respiratory tract-derived epithelial cells. In a study by Krzyminska and coworkers, showed the number of bacteria adhered to HEp-2 cells was between 17.7×10^5 and 28.5×10^5 CFU however other bacteria as the non-pathogenic *E. coli* K-12 C600 showed adhesion 6.1×10^5 CFU.[19]

In this study, there were also an association between the binding capacity and the resistance. MDR isolates might have a higher binding capability than the sensitive one (e.g 6.7×10^4 MDR isolates adhesion in HEp-2 cells vs.1.1×10^4 in sensitive isolates). In a study by Lee et al in 2008 observed that the high colonizing and adhering capacity of *A. baumannii*, combined with its resistance to multiple drugs, will contribute to the organism's survival and further dissemination in the hospital setting.[20]

The effective concentration for OmpA to cause reduction to MTT dye in A549 cells was approximately slightly higher than used in HEp-2 cells. A similar finding was previously reported that using different cell lines could affect the concentration of OmpA necessary for inducing cell death. [21] Other studies suggested also that 20 µg/ml *A. baumannii* OmpA caused a 10% reduction in the conversion of MTT to formazan crystals in incubated A549 cells, and 40 and 80µg/ml of OmpA produced a greater than 75% reduction. Further, a concentration of 3µg/ml of OmpA resulted in approximately 50% cell death and 6 µg/ml produced approximately 100% cell death in HEp-2 cells.[14] Choi et al suggested that ≥6ug/ml of OmpA purified from *A. baumannii* enhance the cytotoxicity to the epithelial cells.[4] Other study stated that the concentration of the protein ≤20µg/ml do not cause cell death. However, 50 and 100 µg/ml of protein concentration might induce cell death.[22] In the present work, 5to99 µg/ml of protein concentration induces apoptosis according to the cell line used.

The hallmarks of apoptotic cell death are mainly through activation of caspases as an absolute marker of cell death, appearance of distinctive cellular morphology with preservation of organelles and cell shrinkage.[23]

In the current study, apoptosis occurred to the cells treated with AbOmpA are correlated with caspase activation (e.g 5.1ug/ml protein which is lowest concentration activate caspase3). Smani et al observed that exposure of A549 cells to resistant strains of *A. baumannii* induce the activation of calpain and caspase-3.[23] Rumbo et al mentioned also that AbOmpA porin induces apoptosis in human epithelial cells.[5]

In conclusion, the most alarming finding in our study along with other studies is the importance of binding capacity of *A. baumannii* to respiratory tract-derived epithelial cells. The results shed a light on the role of OmpA in the MDR *A. baumannii* isolates pathogenesis as it causes apoptosis to the respiratory cells through caspase dependent pathway mechanism. In this work, we could not demonstrate an association between antimicrobial resistance and the role of AbOmpA; however, the results support the idea that OmpA of MDR strains induced the apoptosis through caspase activation. One study stated that AbOmpA is relatively impermeable to antimicrobial agent when compared with other gram negative bacteria and is contributor to intrinsic antimicrobial resistance.[24] The results demand further studies to investigate the clinical relevance of OmpA expression in MDR isolates.

ABBREVIATIONS

A. baumannii: *Acinetobacter baumannii*
API: Analytical profile index
OmpA: Outer membrane protein A
ICU: intensive care units
PBS: Phosphate Buffer saline
SDS: Sodium Dodecyl Sulphate
MDR: Multidrug resistant
LPS: lipopolysaccharide
AbOmpA: Outer membrane protein A of *Acinetobacter baumannii*

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