

## INDUCTION OF EPITHELIAL CELL APOPTOSIS BY *ACINETOBACTER BAUMANNII* A424

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**ABSTRACT:** *Acinetobacter baumannii* is considered as highly contagious pathogen, it can cause serious infections in the Skin, lungs, blood, and brain. It may also cause burn, urinary tract and wound infections. It can be spread by direct contact and may be found on skin or in food, water, or soil. It may also be found in hospitals. Learning how dose *A.baumannii* effect on cells and program of cell death has been a major research goal for researchers. In current study, we chose *Acinetobacter baumannii* A424 strain as a model for cell apoptosis since little information are available for this strain. Live bacteria were more likely to produce epithelial cell death after few hours of incubation. Killed and filtrate form of this strain gave moderate apoptotic activity in HACAT cell, degraded DNA of apoptotic cell showed approximately 140 pb in size. In conclusion the ability of A424 strain of *Acinetobacter baumannii* to cause cell apoptosis as well as other o9ther characteristics like multidrug resistance activity, biofilm formation and survival in desiccation (data not shown) may reflect the virulence characteristics of this opportunistic strain which may infect ill or severely wounded patients.

**KEYWORDS:** *Acinatobacter baumannii*, Cell apoptosis, virulence factor.

## INTRODUCTON

In recent years *Acinetobacter baumannii* has increasingly been responsible for human nosocomial infections. *Acinetobacter* is a killer bug in some departments in some hospitals. As a hospital pathogen, *A. baumannii* mainly affects patients in the intensive care unit (ICU), including burn patients, trauma patients, and patients requiring mechanical ventilation. Also, any immunocompromised patient or anyone who has an underlying disease, such as chronic lung disease or diabetes, is at an increased risk for *A. baumannii* infection. These bacteria were nomnanted recently as “Iraqibacter” because of its origin in military hospitals in Iraq and its persistence among veterans. (1,2,3) Interest in *Acinetobacter*, from both the scientific and public community, has risen sharply over recent years.(4,5,6) .The full genome sequencing of *Acinetobacter baumannii* shows that this organism harbors a remarkable number of putative virulence-associated genes and elements homologous to the *Legionella*/*Coxiella* type IV secretion apparatus(7,8). Several virulence determinants, such as biofilm formation, adherence and ability

to invade host cells were detected in *A.baumannii* (9). Some infections associated with *A.baumannii* include ventilator-associated pneumonia, skin and soft-tissue infections, secondary meningitis, urinary tract infections, wound and blood stream infections, endocarditis, intra-abdominal abscess, and surgical site infections.10. In this study we the ability of *A.baumannii* A424 to cause apoptosis in HACAT epithelial cells.

## **MATERIALS AND METHODS**

### **Bacterial strain and growth conditions**

*Acinetobacter baumannii* A424 strain was obtained from Dr.K.Rajakumar University of Leicester (UK). Prior each experiment, a single colony of baumannii A424 was inoculated into 5ml Luria broth and grown at 37C with shaking for 18 hour. Bacterial culture was washed three times with buffer saline (PBS at PH 7.2) inoculated to cell line culture (whole cell), killed bacteria were also used by exposing bacteria to 60C for 15 min. filtrate culture was prepared by inoculation *A.baumannii* A424 to LB medium, the cultured bacteria were centrifuged and the supernatants were filtrated using 0.22µm. references

#### **Cell line culture**

Human HACAT epithelial cell was grown in DMEM medium supplemented with 10% fetal calf serum (FCS), 100U of penicillin per ml and 100mg streptomycin per ml at 37C in 5%CO<sub>2</sub>. Cells were observed daily under light microscope.

### **Induction of cell apoptosis**

HACAT epithelial cells were treated with *A.baumannii* A424 (whole cells, killed bacteria and filtrate). Cells were observed after (2, 4, 8, 16, 24 and 48h). 10<sup>8</sup> CFU were used for whole bacteria Reference. Infected cells were incubated at 37C with 5%CO<sub>2</sub>.

### **Detection of cell death**

a.Trypan blue uptaking: After 24h of incubation, cells apoptosis were examined for using trypan blue stain. Cells were washed gently three times with PBS, and stained with 0.4% for 5 min. cells were examined by inverted phase contrast light microscopy and cells count were detected using hemocytometer. (11)

b.DNA fragmentation was carried out according to the protocol of (12) with slight modification, DNA from The adherent HACAT cells were harvested and analyzed for DNA fragmentation. Cells were collected by scrapping, washed in PBS and treated with Triton X100 (source or company), cells were collected and centrifuged at 3500rpm for 15min. cells were re-suspended and treated with RNase for 37C for 1 hour. 20 µg/ml of protease K was added (50C for 2 hours). DNA extraction was extracted with phenol, chloroform, isoamyle alcohol (25, 24, 1), and centrifuged, to precipitate DNA, supernatant was treated with equal volume of isopropanol with overnight

incubation at -20°C. Sample was centrifuged (4°C, 15min) and treated with TBA (Tris boric acid), analysis was done on a 1.5% agarose gel.

**Electron microscopy:** Infected HACAT epithelial cells were grown in 35mm culture dishes at concentration of  $10^6$  cells/ well. *A. baumannii* A424 was infected to HACAT cells with MOI 100, 50 and incubated for 18h (7), the cells were washed four times with PBS and harvested by using tryton X100 and re-suspended in appropriate fixative. Samples were sent to the electron microscopy section at the University of Leicester for further investigation.

## RESULTS

Learning how *A. baumannii* effect on cells and program of cell death has been a main goal for researcher, In the current study we chose *A. baumannii* A424 as a model for this bacterium and according to the knowledge of Author, no information are available about cell apoptosis by this strain of Acinetobacter.

### Induction of HACAT cell apoptosis:

To investigate the ability A424 of Acinetobacter strain to invade HACAT epithelial cells, whole cell, killed and filtrate form was applied. (Fig 1), whole cell caused noticeable apoptotic activity after 24h and 90% apoptosis was detected after 48h., killed and filtrate bacteria had less apoptotic activity after 24h than whole bacteria. rther processing.

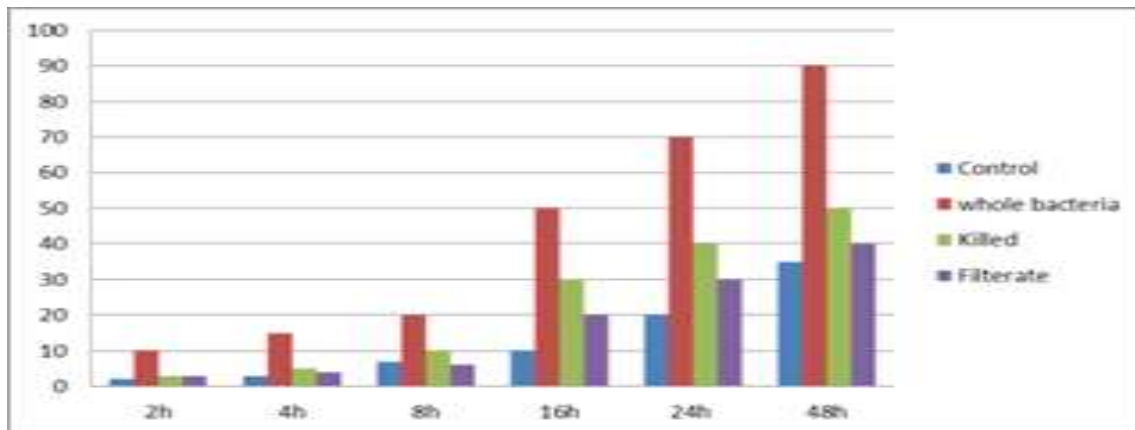


Fig.1. Effect of live bacteria killed and filtrate on viability of HACAT Epithelial cell. Cells were co-cultured with *Acinetobacter baumannii* A424 (2,4,8,16,24,48h)



A. Healthy cell

B. after 24 hour

C. After 48hour

Fig.2. A. illustrates the morphology of healthy HACAT epithelial cell, B,C infected cells after 24, 48 h

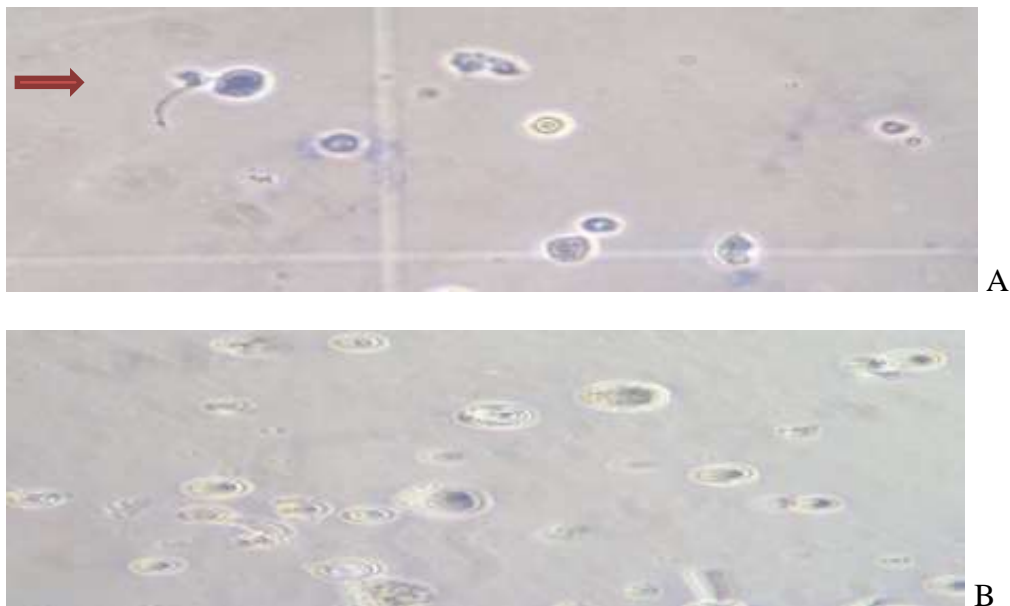


Fig.3. A trypan blue positive cells after 24h B .control cells.

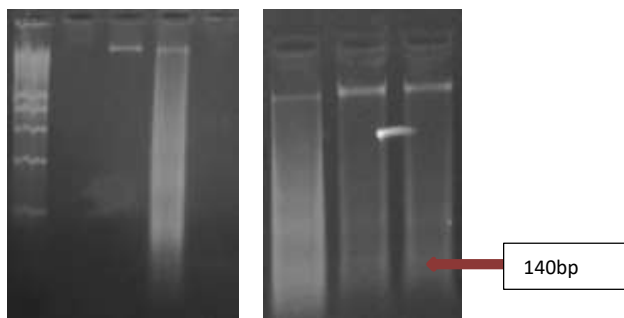


Fig.4. DNA fragmentation of cell after infection with *A.baumannii* A424

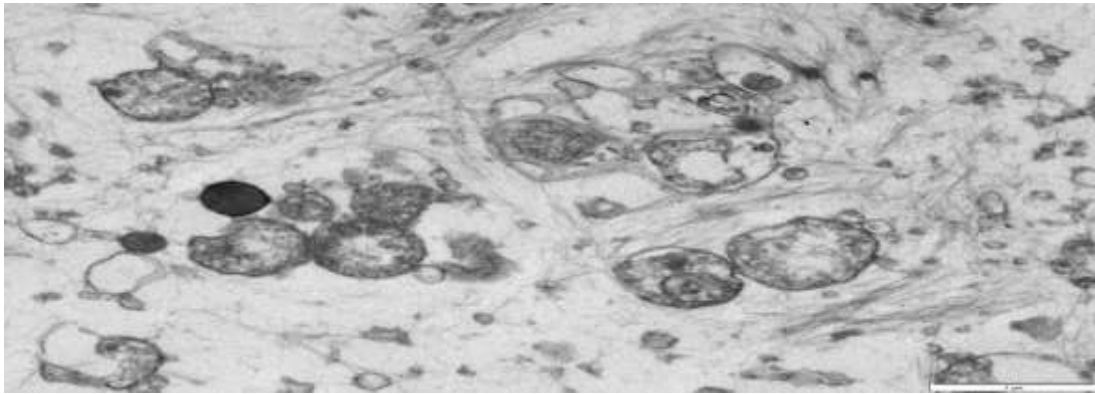


Fig.5. Electron microscopy for *Acinetobacter baumannii* A424

## DISCUSSION

Multidrug-resistant *Acinetobacter baumannii* is a formidable threat and associated with wide spectrum of infectious diseases including nosocomial infections, community-acquired and those following war or natural disaster especially to military personnel with war wound. The treatment has become difficult, not only because the bacterium can develop extensive antimicrobial resistance but because it also forms biofilms that are resistant to host defense as well as causing host cell apoptosis.(13,2) .In current study *Acinetobacter baumannii* A424 strain was chosen as a model to see the ability of this strain to induce HACAT cells apoptosis, data illustrated that whole bacteria had a strong apoptotic activity after 24h, less among killed and filtrate form, this indicates that living cell of A424 strain may contain apoptosis inducing molecule(s), that may activate the program of cell death and increases the virulence ability of this bacteria, Although other virulence factors were confirm for this strain including biofilm formation, survival in desiccation and multidrug resistant activity (data not shown),it has been reported that the apoptosis of epithelial cells was induced by various components of bacteria. (14). Activation of casebase is an important step for cell apoptosis, investigations demonstrated that the precursor form of casepase 3 is cleaved by live *A.baumannii* to produce active form by live bacteria (5). The ability of bacteria to induce host cell apoptosis is considered to be an important bacterial virulence mechanism.(3). Bacteria may even up-regulate the endogenous receptor/ligand system that induces apoptosis, generally when the bacteria are bound to the host cell.( 15), it was noticed in current study that HACAT apoptosis was started at early stage for whole lived bacteria. A study carried out by (16-18) demonstrated that Omp38 is considered as potent cytotoxin that induces apoptosis of epithelial cells in *A.baumannii* and this may explain the strong effect of live bacteria on cell apoptosis. In current study cell apoptosis was also detected with filtrate form of A424 baumannii this finding suggests that this strain may produce certain products that mediate cell apoptosis. (19,5).

In conclusion the ability of A424 strain of *Acinetobacter baumannii* to cause cell apoptosis as well as other characteristics like multidrug resistance activity, biofilm formation and survival in

desiccation (data not shown) may reflect the virulence characteristics of this opportunistic strain which may infect ill or severely wounded patients.

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