Pathogenesis of *Acinetobacter* spp.:

Resistance and Virulence Converge

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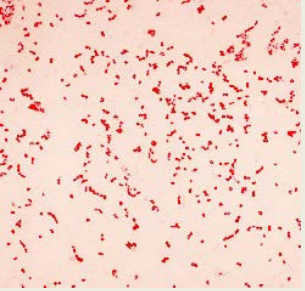
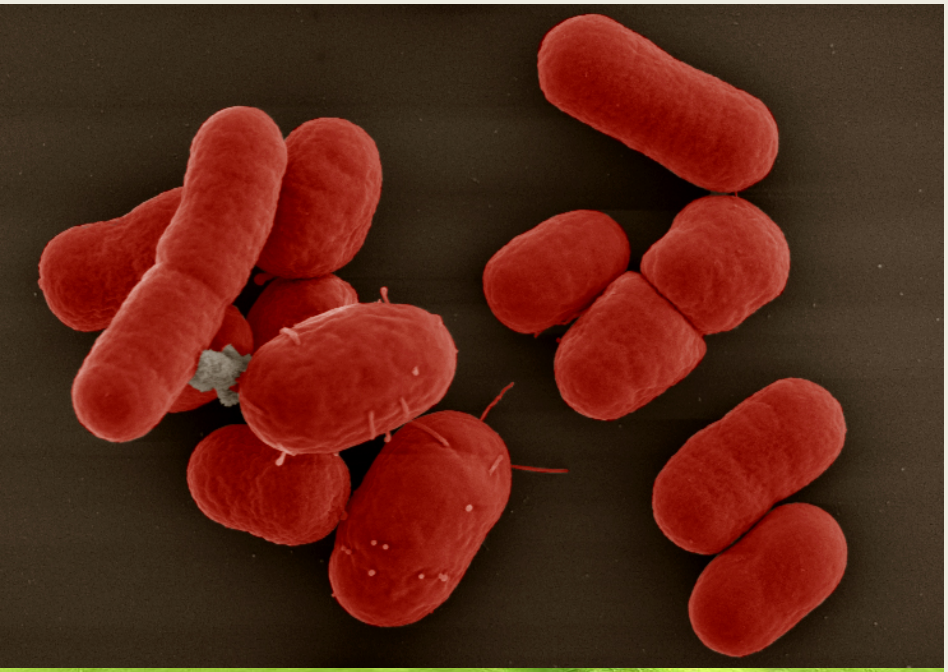
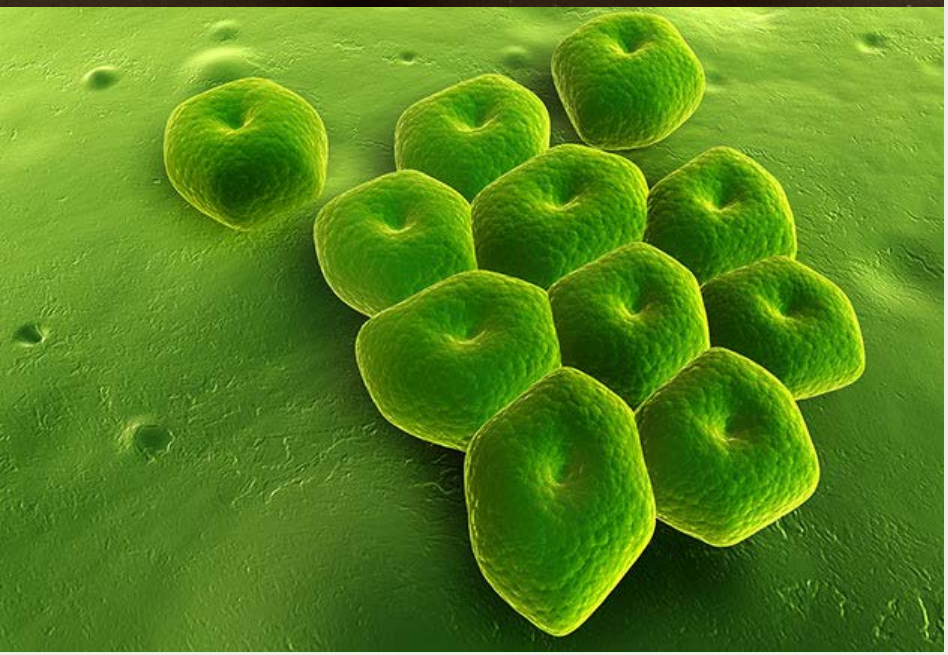


Acinetobacter spp.

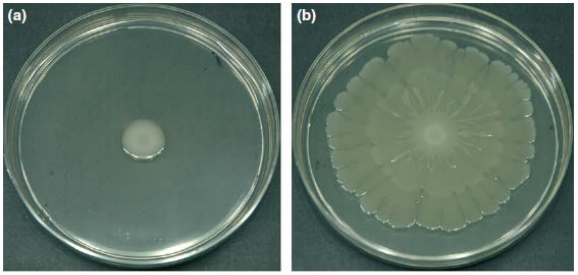
- **Among the most <u>complex</u> of pathogens (a "sordid past")**
  - ***Mima polymorphia*, *Herella vaginicola*, *Bacterium anitratum*, *Moraxella*, B5W**
- **Highly diverse, oxidase-(+) and (-) , Gram-negative coccobacilli.**
- **> 50 species --the majority are nonpathogenic**
- **Species causing infection : *A. baumannii**, *A. calcoaceticus* and *A. lwoffii* , *A. haemolyticus*, *A. johnsonii*, *A. junii*, *A. nosocomialis*, *A. pittii*, *A. schindleri*, *A. ursingii*, and *A. seifertii* (an emerging pathogen in Asia)**
- α + κίνητο + βακτηρ(ία) =**"*akineto*" = "non motile rod"-really?**
- **"Warm and wet"**






BUACH

“Non-motile”


(a)
(b)
(c)
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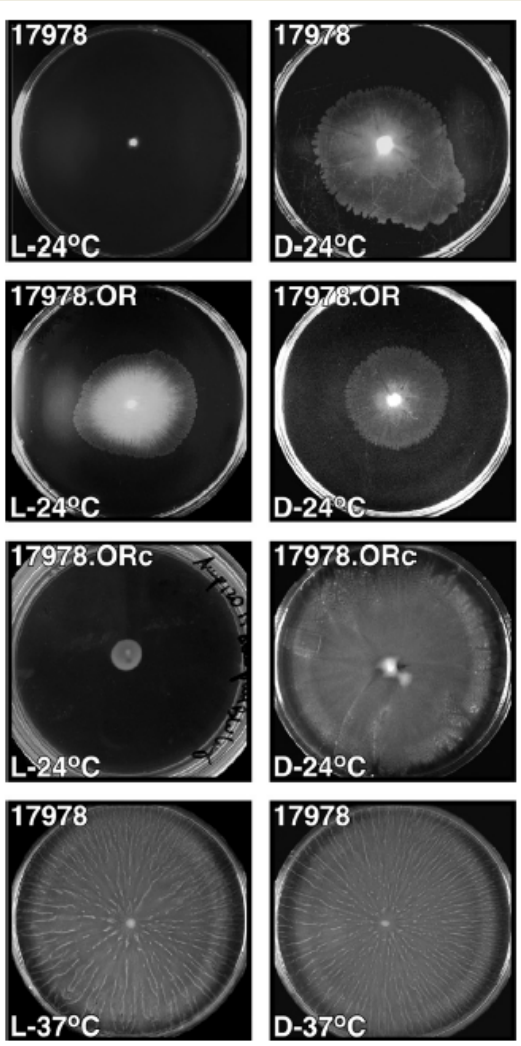
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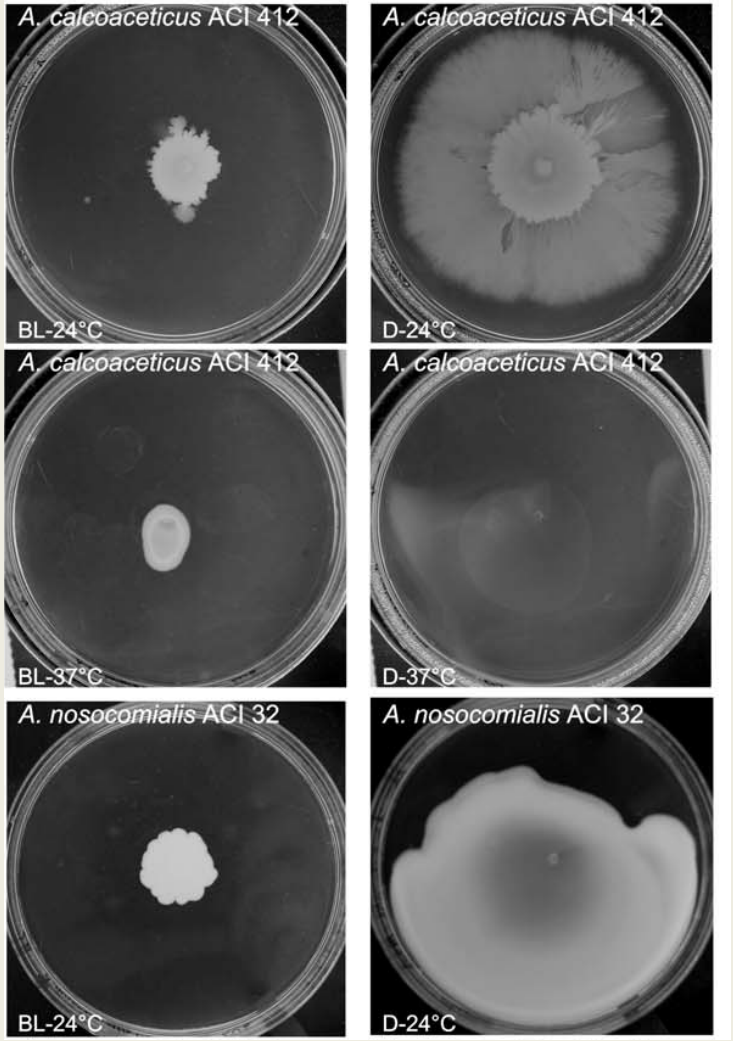

A. calcoaceticus ACI 412
BL-24°C
A. calcoaceticus ACI 412
D-24°C
A. calcoaceticus ACI 412
BL-37°C
A. calcoaceticus ACI 412
D-37°C
A. nosocomialis ACI 32
BL-24°C
A. nosocomialis ACI 32
D-24°C

Spectrum of Human Infections- increased attributable mortality

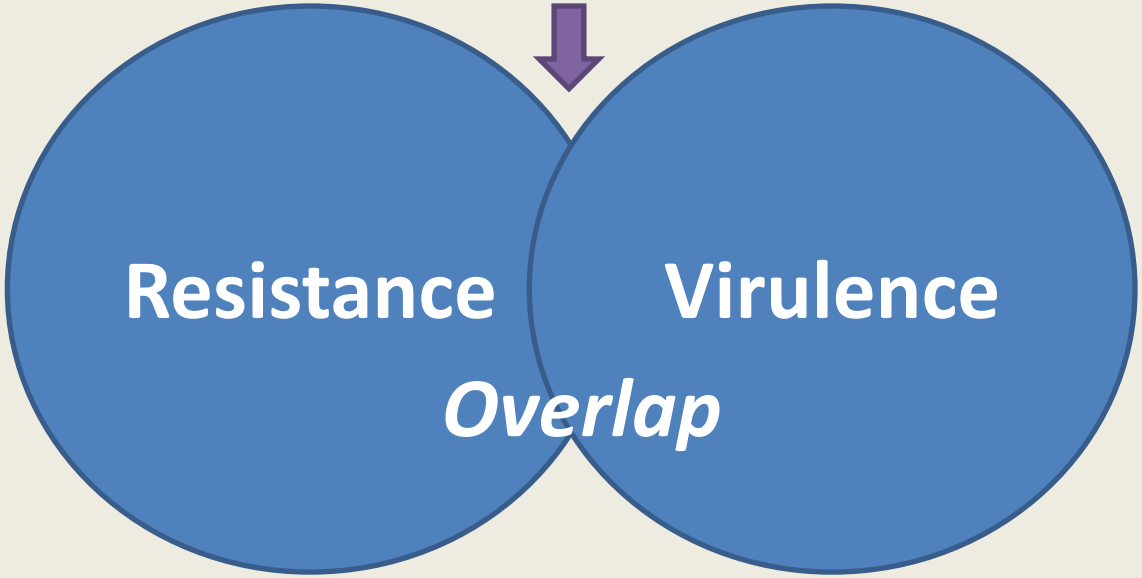
- **Hospital acquired, health care associated and community acquired**
  - **Pneumonia**
    - **mechanical ventilation (40-70 % mortality)**
    - **community acquired (40-60 % mortality, alcohol use)**
  - **BSI**
  - **Burn**
  - **SSTI (Iraq experience)**
  - **Meningitis (33% mortality)**
  - **Osteomyelitis**
  - **Endocarditis**

The mark of a successful pathogen--resistance + virulence

Resistosome* of *A. baumannii

Virulosome* of *A. baumannii

Factors??? Balance???



Remarkable capacity of A. baumannii to acquire and rearrange genetic determinants that play a critical role in its pathobiology

How do we know *Acinetobacter* spp. are virulent?

What are the clues?

What does this mean?

Observations
Finding Models
Defining Virulence Traits

AJIC brief reports

The early days at WRAMC….
Virulence and resistance?….

Antibiotic resistance determinants in *Acinetobacter* spp and clinical outcomes in patients from a major military treatment facility

Federico Perez, MD,[a,b] Andrea M. Hujer, BS,[b] Edward A. Hulten, MD,[c] Joel Fishbain, MD,[c] Kristine M. Hujer, BS,[b] David Aron, MD,[b] Katherine Thweatt, PhD,[b] Curtis J. Donskey, MD,[b] and Robert A. Bonomo, MD[b,d]
Cleveland, Ohio, and Washington, DC

Antibiotic-resistant phenotypes and genotypes in *Acinetobacter* spp with clinical outcomes and characteristics in 75 patients from a major military treatment facility.

Carbapenem resistance associated with the need for mechanical ventilation.

Table 1. Phenotypes and outcomes

	Carbapenem		
Outcome	**R**	**S**	***P* value**
No. of isolates	19	56	
Intubation	17	34	.020*
No intubation	2	22	
HAI	5	19	.545
Non-HAI	14	37	

	Mean	SD	Mean	SD	*P* value
No. days into outbreak	481.32	206.63	369.14	197.53	.005*
No. of antibiotics	6.68	3.96	5.52	3.78	.182
Duration of antibiotics, days	45.53	40.11	34.82	36.38	.395
No. of antibiotic changes	6.68	4.41	4.70	4.54	.068
Days ICU stay	20.21	34.88	18.52	35.01	.321
Days inpatient	66.68	64.51	47.73	40.19	.535

R, resistant; *S*, susceptible; *HAI*, hospital-acquired infection.
*$P < 05$.

bla$_{OXA-23}$ associated with mechanical ventilation, longer hospital and ICU stay, complexity, duration, and changes made to antibiotic regimens.

Table 2. Genotypes and outcomes

	bla$_{OXA-23}$		
Outcome	**Positive**	**Negative**	***P* value**
No. of isolates	8	67	
Intubation	8	43	.041*
No intubation	0	24	
HAI	3	21	.729
Non-HAI	5	46	

	Mean	**SD**	**Mean**	**SD**	***P* value**
No. days into outbreak	488.38	215.70	386.72	202.02	.056
No. of antibiotics	8.13	3.18	5.54	3.83	.026*
Duration of antibiotics, days	71.63	45.16	33.46	34.53	.010*
No. of antibiotic changes	8.75	3.20	4.78	4.54	.005*
No. days ICU stay	35.88	50.21	16.93	32.37	.040*
No. days inpatient	90.13	62.54	48.04	44.18	.036*

NOTE: Positive refers to a present gene. Negative refers to an absent gene.

HAI, hospital-acquired infection.

*$P < 05$.

bla$_{OXA-23}$ - a marker?

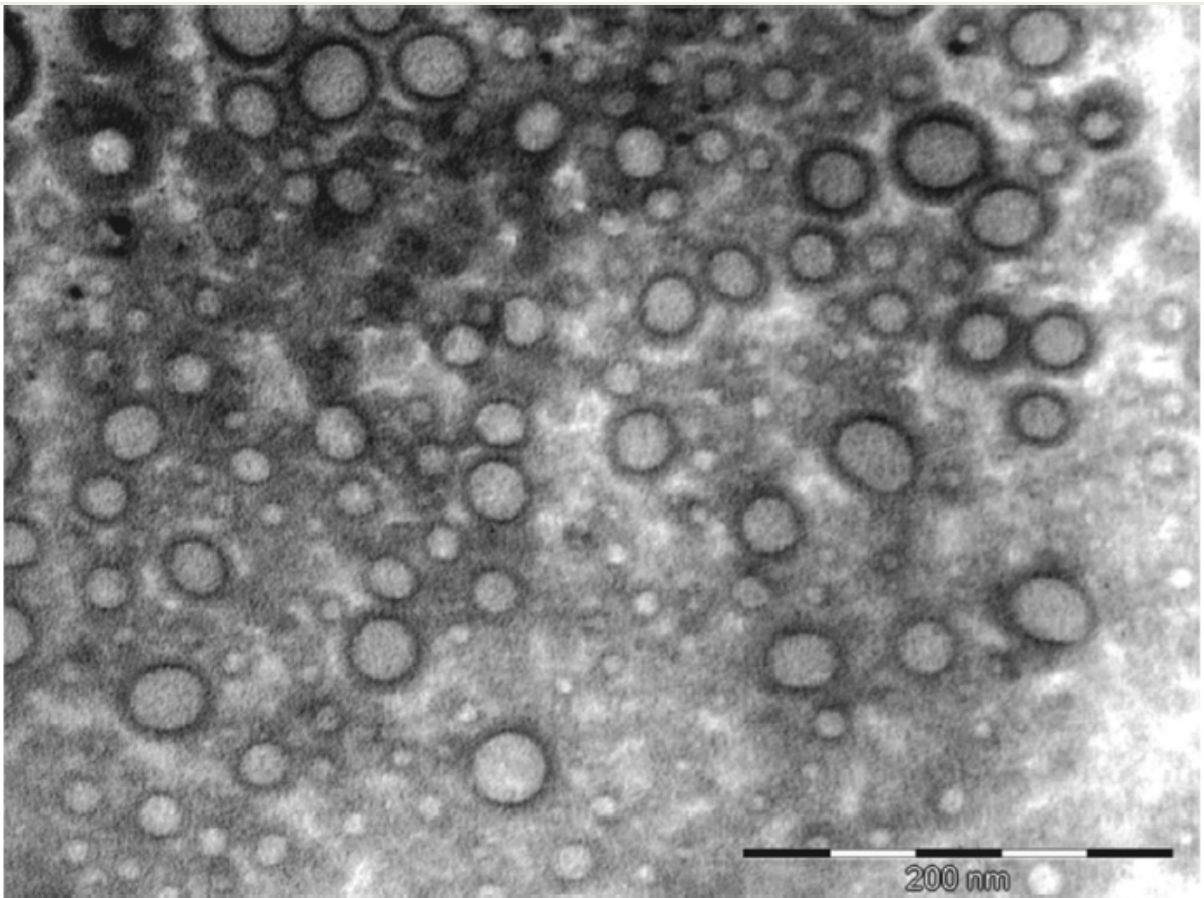
- **Infections caused by *A baumannii* that harbor *bla*$_{OXA-23}$ may be associated with more difficult to treat clinical conditions.**
- **The associations….The number of antibiotics used, duration of antibiotic use, and number of changes in the antibiotic regimen.**
- **Is there a link within the bacterium of proteins in the periplasmic space and cytosol and other virulence factors??**

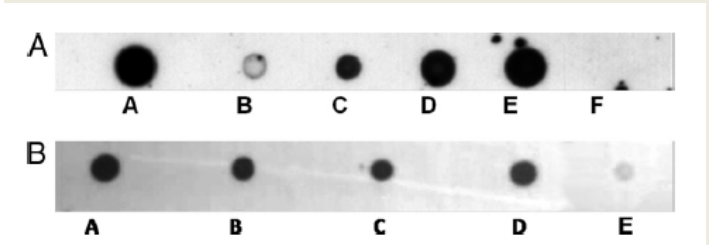
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2011, p. 3084–3090 Vol. 55, No. 7
0066-4804/11/$12.00 doi:10.1128/AAC.00929-10

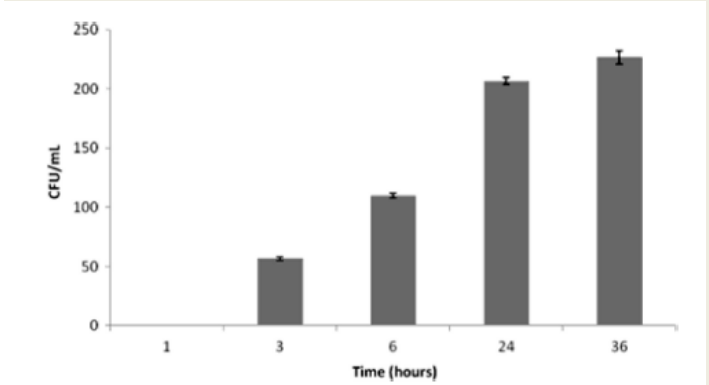
A mechanism of horizontal gene transfer

Horizontal Transfer of the OXA-24 Carbapenemase Gene via Outer Membrane Vesicles: a New Mechanism of Dissemination of Carbapenem Resistance Genes in *Acinetobacter baumannii*[▽]

Carlos Rumbo,[1]† Esteban Fernández-Moreira,[1]† María Merino,[1]† Margarita Poza,[1] Jose Antonio Mendez,[1] Nelson C. Soares,[1] Alejandro Mosquera,[2] Fernando Chaves,[3] and Germán Bou[1]*







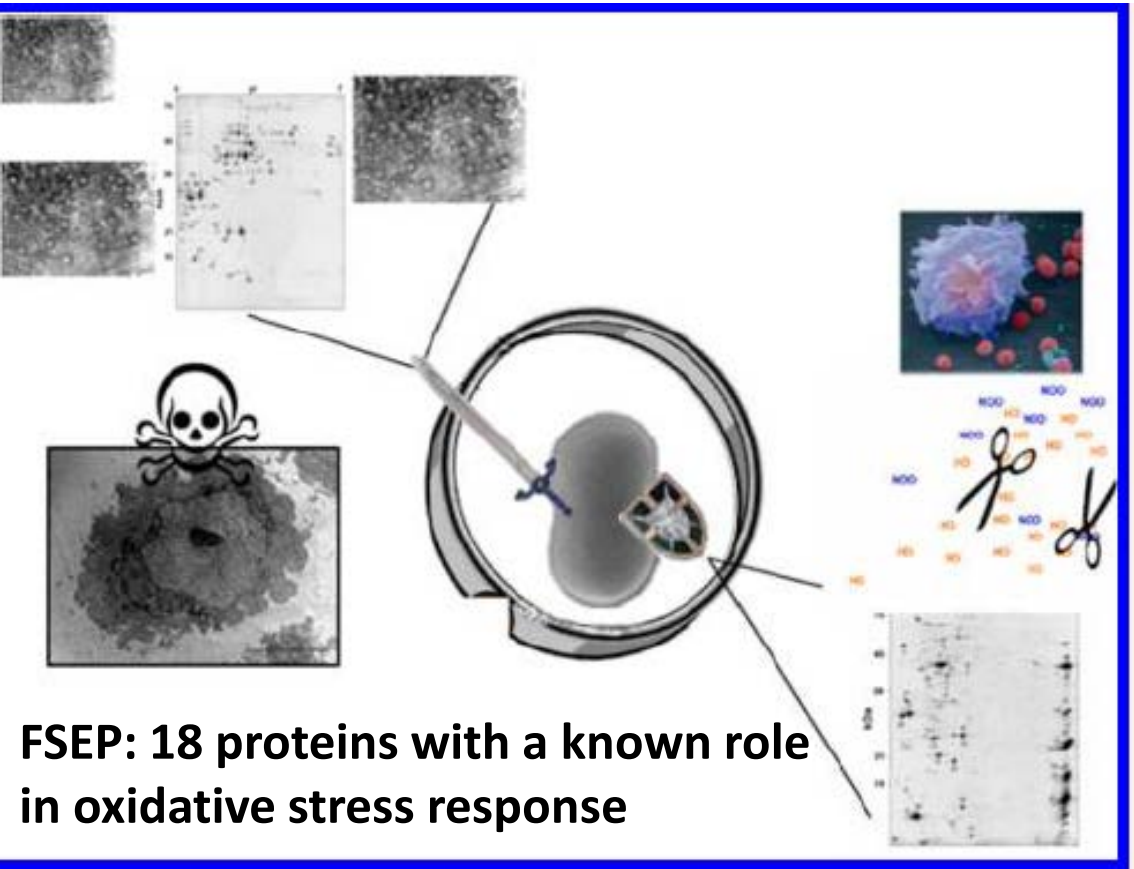
Journal of proteome research

(OMVs) and freely soluble extracellular proteins (FSEPs)

pubs.a

Extracellular Proteome of a Highly Invasive Multidrug-resistant Clinical Strain of *Acinetobacter baumannii*

Jose Antonio Mendez,[†,‡] Nelson C. Soares,[†,‡] Jesús Mateos,[§] Carmen Gayoso,[†] Carlos Rumbo,[†] Jesús Aranda,[†] Maria Tomas,[†] and Germán Bou*[,†]



FSEP: 18 proteins with a known role in oxidative stress response

Of the OMV proteins, 39 were associated with pathogenesis and virulence, including proteins associated with attachment to host cells (e.g., CsuE, CsuB, CsuA/B) and specialized secretion systems for delivery of virulence factors (e.g., P. pilus assembly and FilF)

Models of infection

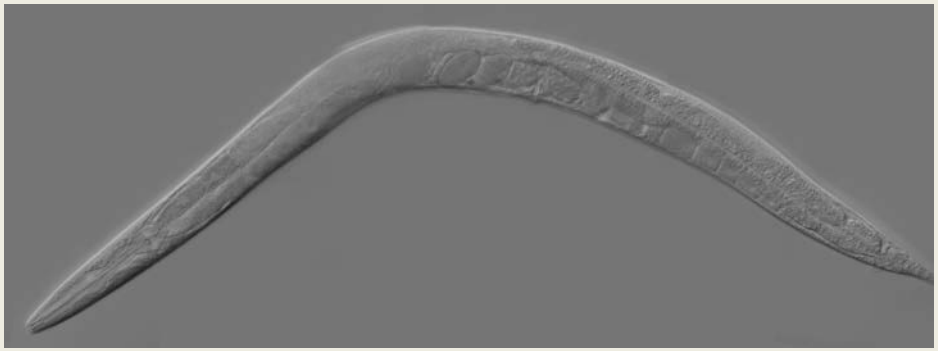
Animal models have been important to identify potential virulence

Elucidate the factors driving the outcome of the interactions between the host and *Acinetobacter* spp.

Caveat

***In vitro* assays, including adherence to human cells (e.g., epithelial cells and/or pneumocytes), cell invasion, and biofilm formation have often lacked correlation with *in vivo* virulence of *Acinetobacter* when studied head to head**

Models of infection to understand pathogenesis



Models of Infection

- **Mice (limitations, some non physiologic)**
  - High inoculum infections (10^9)? Relevance?
  - Neutropenic mouse ? not a common risk factor
  - IP infections-porcine mucin??
  - **Strains of mice intrinsically susceptible: A/J mice demonstrate delayed neutrophil recruitment to the lungs due to diminished CXC chemokine responses to the bacteria, possibly explaining their susceptibility to pulmonary infection**
  - **C3HeB/FeJ strains of mice-unknown**

Models of Infection-II

- **Rats**
  - Pneumonia-better, non-immunocompromised
  - Skin and soft tissue-find virulent strains
  - Wound; Ab5075 and cyclophosphamide
  - Meningitis
  - Osteomyelitis
  - Endocarditis

Models of Infection-II

- **Non mammalian**
  - *Galleria* (waxy moth larva)
  - *C. elegans*
  - Zebrafish larvae

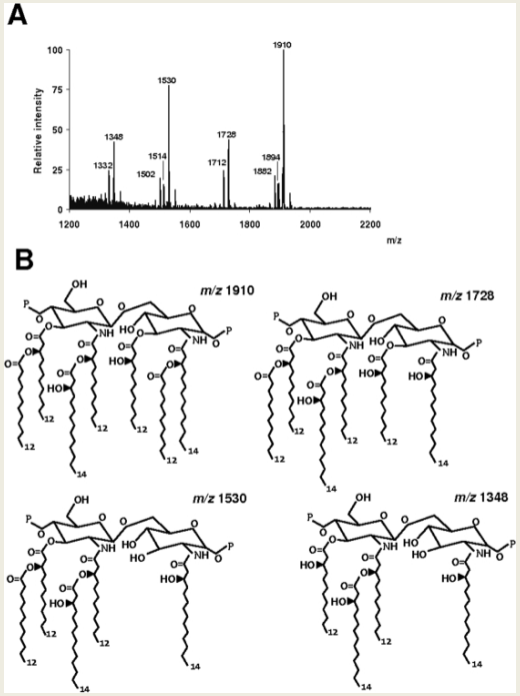
Models lead us to define Virulence + Determinants

- Persistence in dry environments / resists desiccation
- Motility
- Ethanol
- RecA
- Biofilm (adherence mechanisms)
- Fe acquisition pathways
- activities of polysaccharide membrane and outer membrane protein phospholipases
- alteration in PBPs,
- OMVs

Pilus production mediated by the CsuA/BABCDE usher-chaperoneassembly system is required for attachment and biofilm formation on abiotic surfaces by the A. baumannii ATCC 19606T

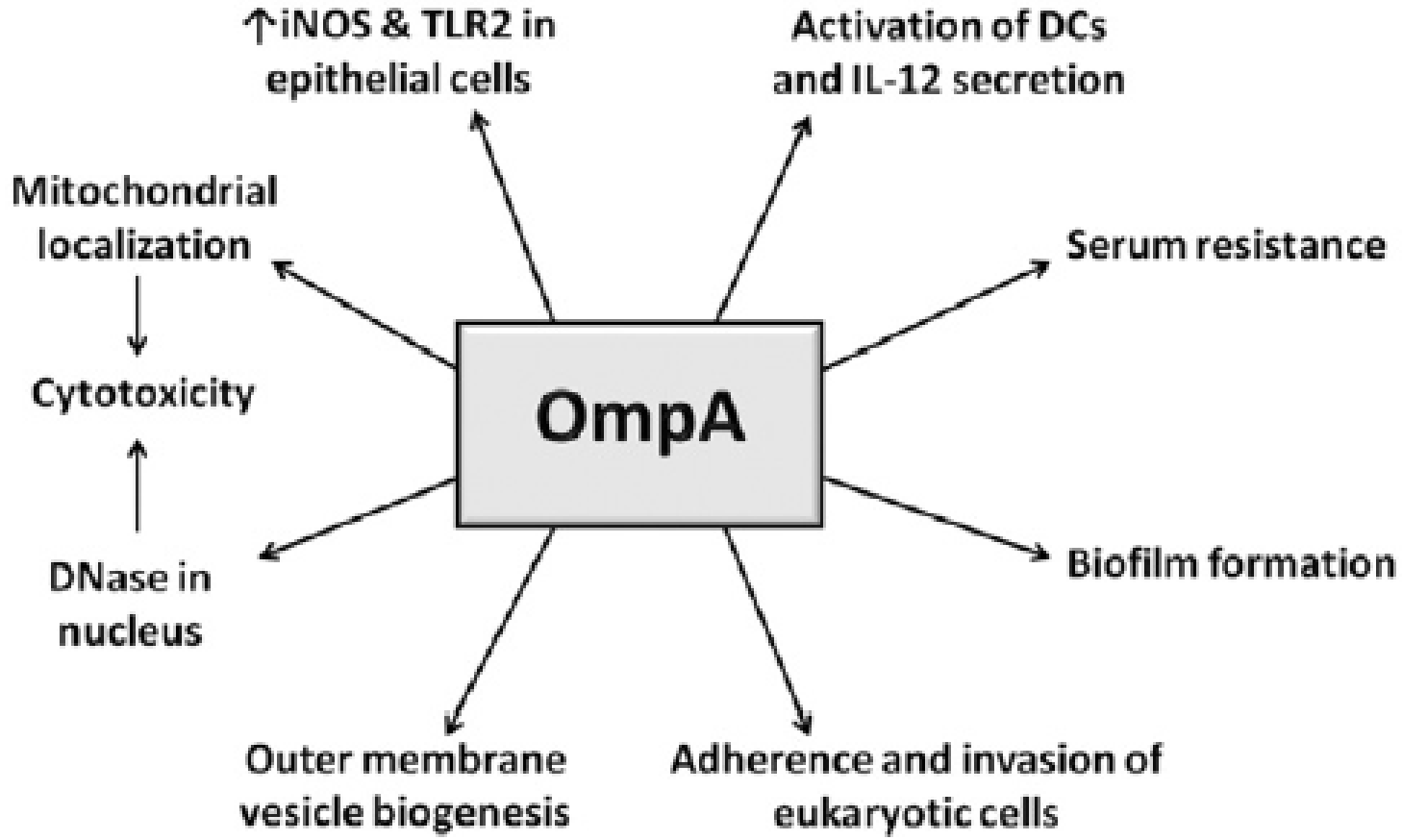
Central Role of LPS

Evasion of Host Immune resposnse



Triggering of Host Inflammation Response

Central Role of OmpA



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Identified Virulence Factors- complexity begins

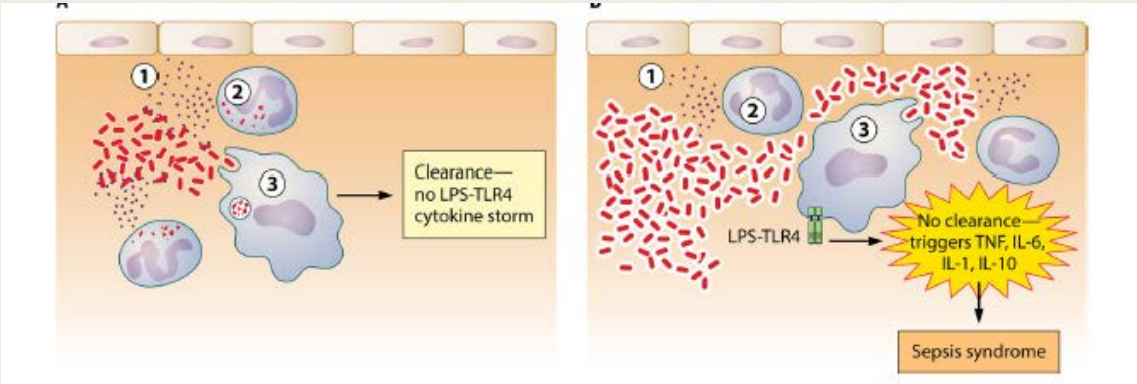
OmpA	CFTR (CiF)	SOD	pmrB	SurA1	OmpA	Zur	pmrA	Omp33
Cpa A	Biofilm gene (LH92_11085)	TonB	lpxACD, pmrB	AdeRS	OMV	ZigA	Acinetobactin (iron siderophore)	MapA
BfmS	KatG and KatE	OXA-40	PLD	*gacA* and *gacS*, *abaI*, *paaA*	LipA	FeoB	Cipro R	Capsule
CarO	NfuA	AbuO	Type VI SS (T6SS)	PTK and EpsA	gspD	pmrB	Acinetobactin	UspA
OprD	EntA	SecA	lpxD	PLD	AdeABC AdeIJK	pmrA	PgILAb (O glycosylation	*pmrB*, *lpxA*, *lpxA*, *lpxC*, *lpxD* (LPS genes)

Immunological defense mechanisms

- Cytokines
- Avoidance of innate effector uptake
- PMNs
- LPS-TLR4 governance of outcome
- Macrophages
- Zn and Mn sequestration
- Iron sequestration

Integrated view of *A. baumannii* virulence

- **Capsule --evades complement and phagocytosis.**
  - **Capsule-primary defense vs. complement-mediated destruction and opsonization, as well as phagocytic uptake**
- **LPS (endotoxin)??**
- **A large infectious inoculum and depletion or reduction of host innate effectors**
- **LPS triggering of TLR4-mediated sepsis.**



Early effective therapy helps the host rapidly clear the bacteria, avoiding subsequent host damage from the sepsis response, whereas early administration of ineffective therapy enables the bacteria to persist at higher blood or tissue bacterial densities, triggering host damage

RESEARCH ARTICLE

Virulence and Resistance converge???

Inhibition of LpxC Protects Mice from Resistant *Acinetobacter baumannii* by Modulating Inflammation and Enhancing Phagocytosis

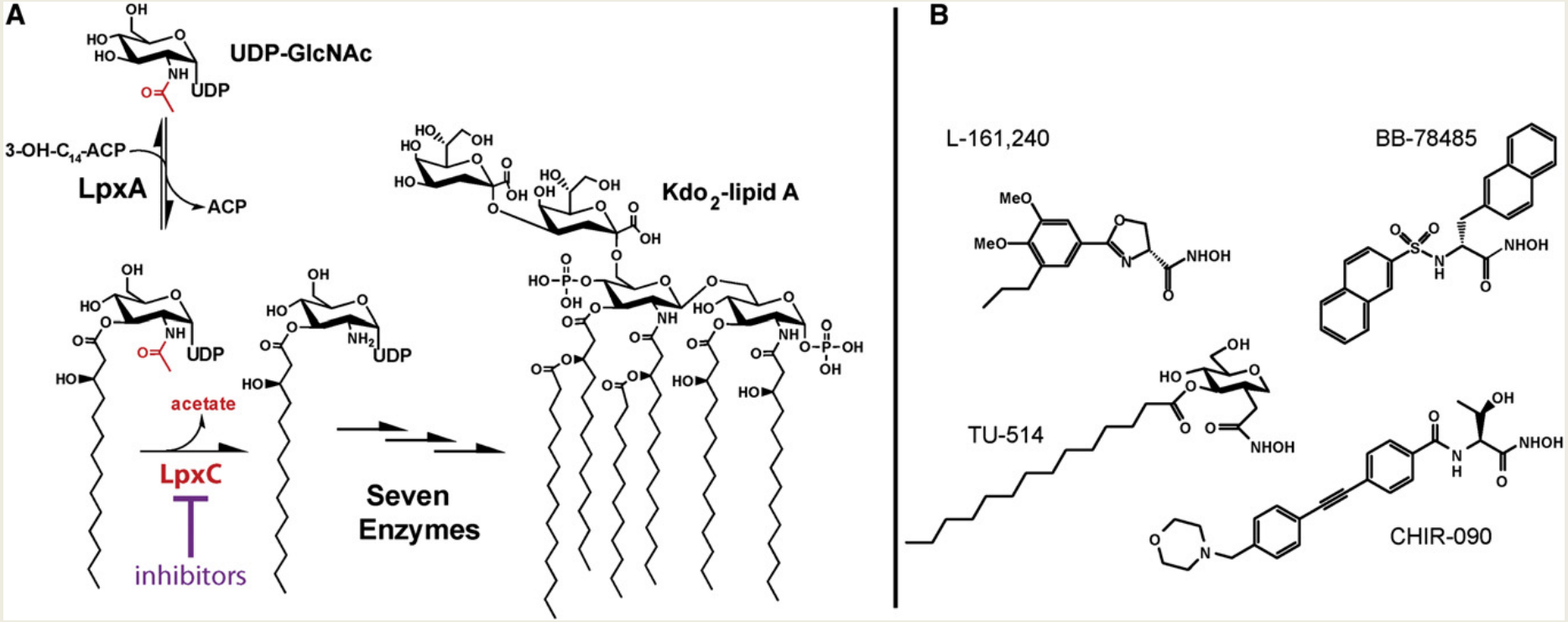
Lin Lin,[a,b] Brandon Tan,[a] Paul Pantapalangkoor,[a] Tiffany Ho,[a] Beverlie Baquir,[a] Andrew Tomaras,[c] Justin I. Montgomery,[c] Usa Reilly,[c] Elsa G. Barbacci,[c] Kristine Hujer,[d] Robert A. Bonomo,[d] Lucia Fernandez,[e] Robert E. W. Hancock,[e] Mark D. Adams,[f] Samuel W. French,[b,g] Virgil S. Buslon,[g] and Brad Spellberg[a,b]

?

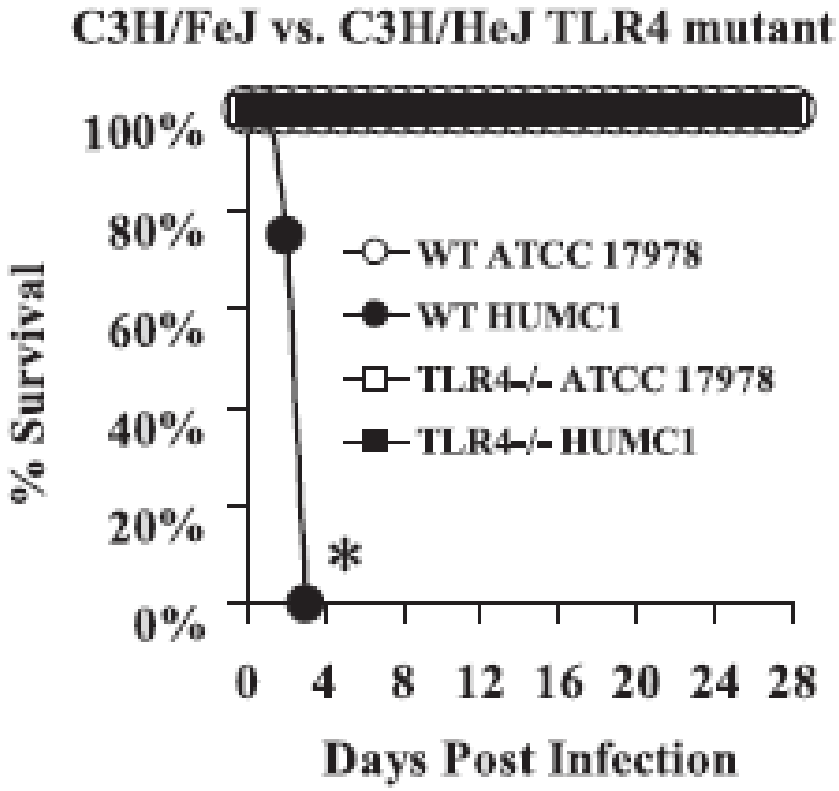
Can an LpxC inhibitor block the ability of bacteria to activate the sepsis cascade, enhanced opsonophagocytic killing of the bacteria, and protected mice from lethal infection.

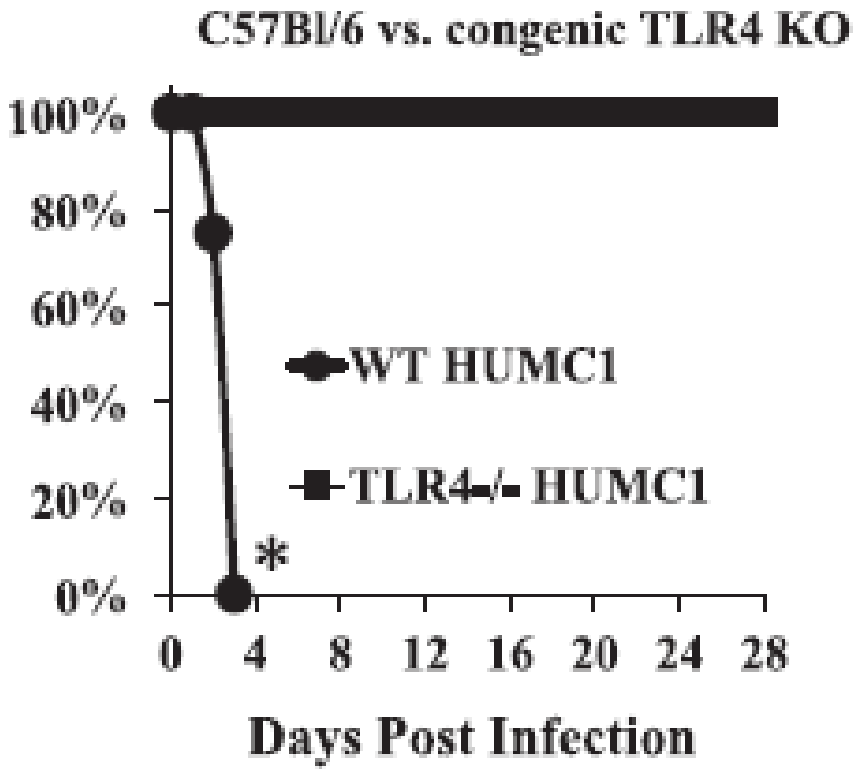
Species-Specific and Inhibitor-Dependent Conformations of LpxC: Implications for Antibiotic Design

Chul-Jin Lee,[1,2] Xiaofei Liang,[3,4] Xin Chen,[3] Daina Zeng,[1] Sang Hoon Joo,[1] Hak Suk Chung,[1] Adam W. Barb,[1,7] Shauna M. Swanson,[5] Robert A. Nicholas,[5,6] Yaoxian Li,[4] Eric J. Toone,[1,2,3] Christian R.H. Raetz,[1] and Pei Zhou[1,2,3,*]

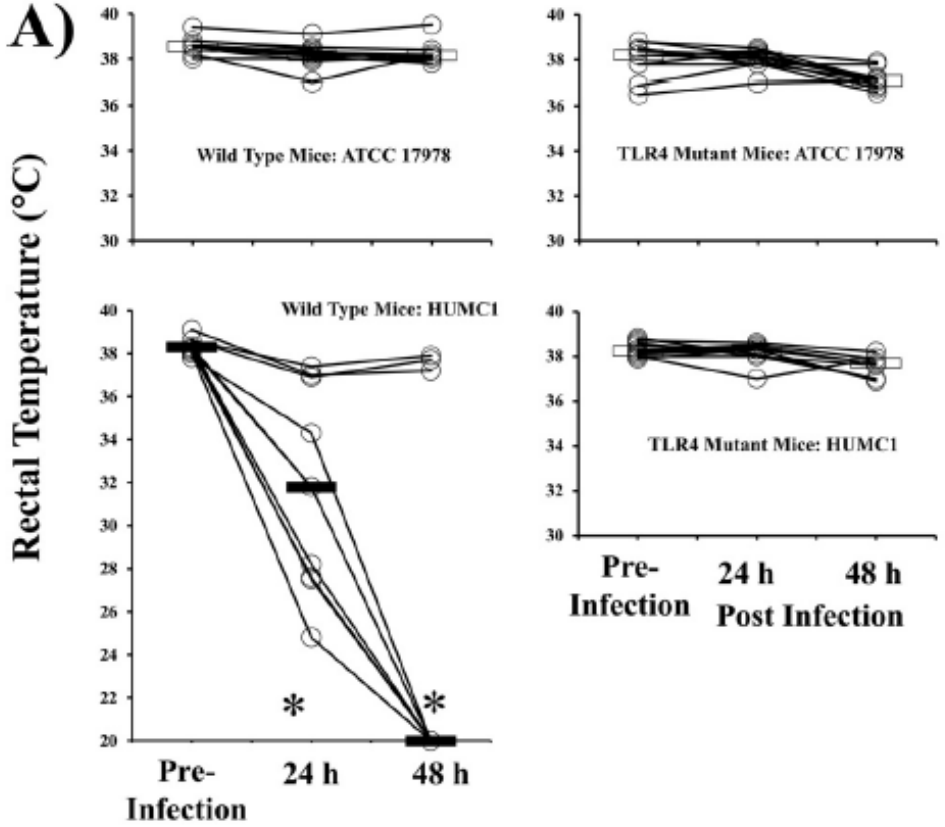


TLR4 is antiprotective against *A. baumannii* bloodstream infection

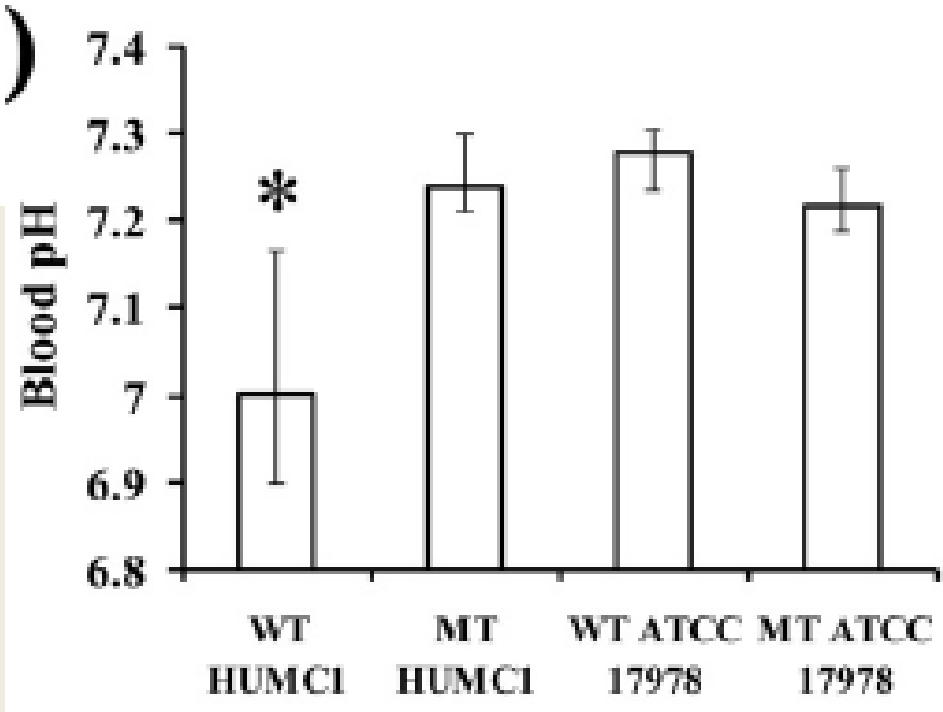


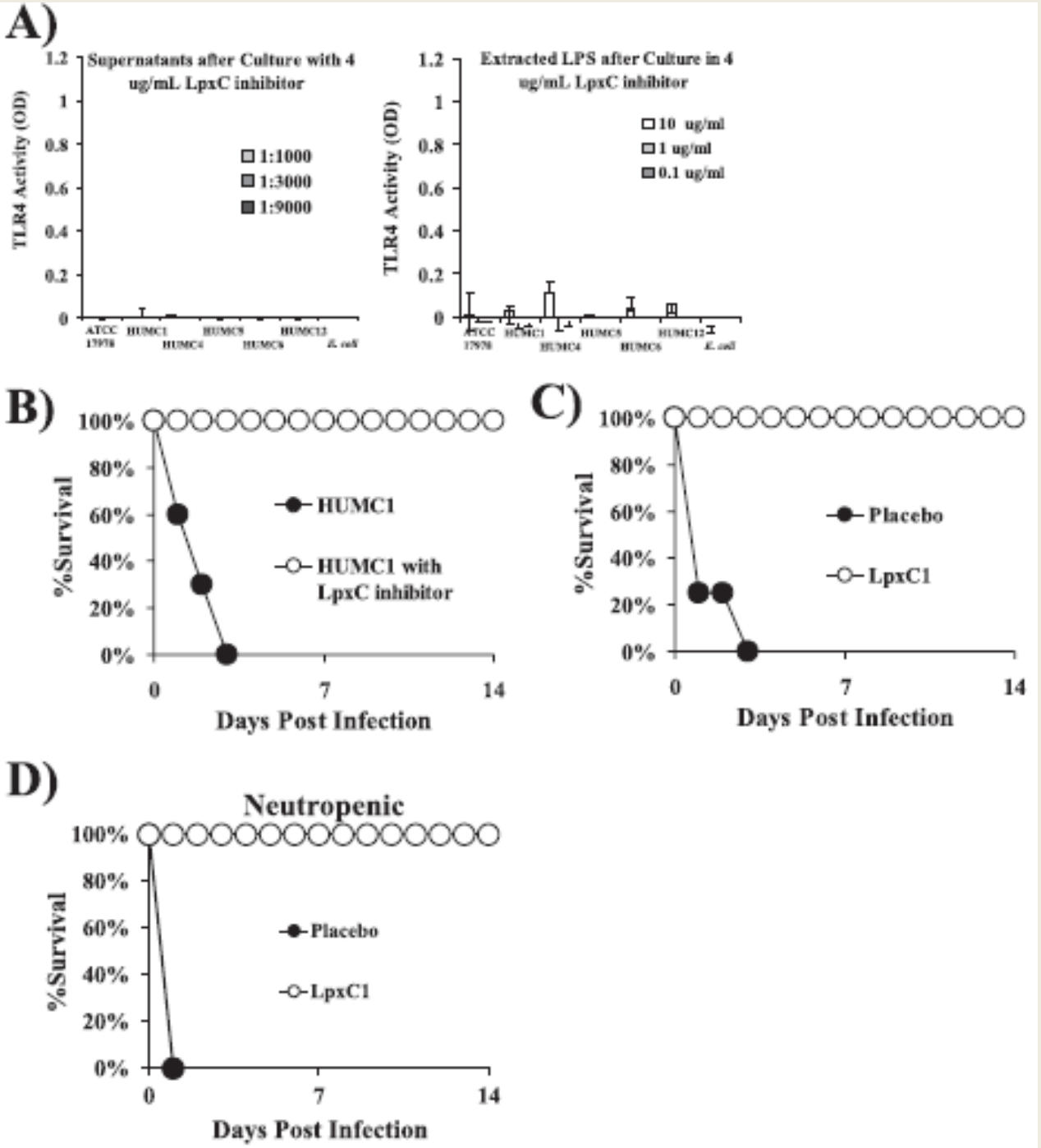


TLR4-mutant mice were not susceptible to and were instead highly resistant to lethal infection caused by *A. baumannii*.



Lethally infected wild-type mice had septic shock, whereas TLR4-mutant mice did not.





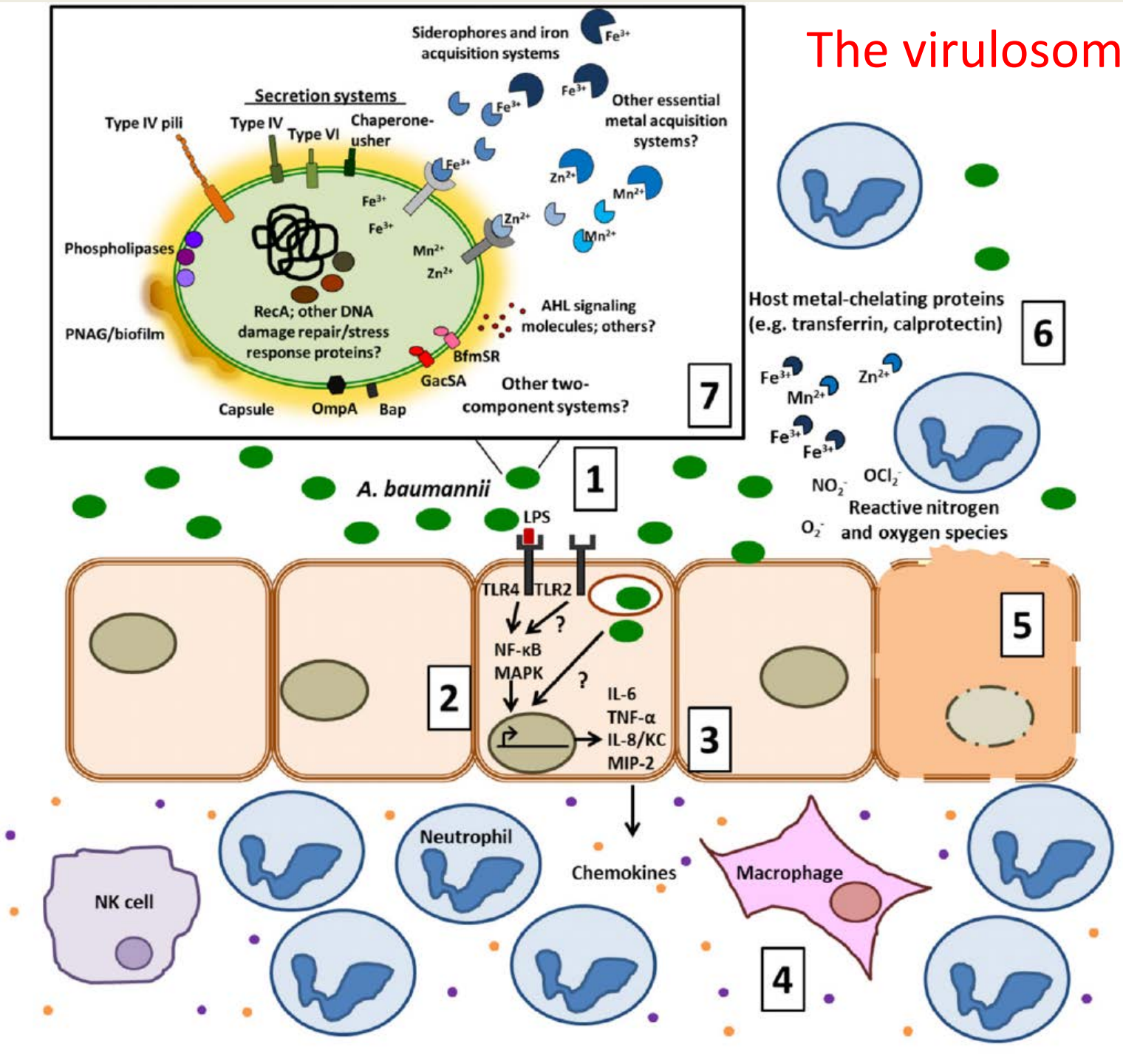
Inhibition of LPS biosynthesis with an inhibitor of LpxC blocked TLR4 activation *in vitro* and abrogated virulence *in vivo*.

A new way of looking at *Acinetobacter*??

- **LPS-mediated activation of TLR4 was a primary pathogenic factor during systemic *A. baumannii* infection, and TLR4 was antiprotective against lethal infection.**

- **Inhibition of LpxC resulted in diminished LPS-mediated TLR4 activation and protected mice from lethal infection despite a lack of *in vitro* susceptibility of the bacteria to the inhibitor by traditional testing.**

The virulosome



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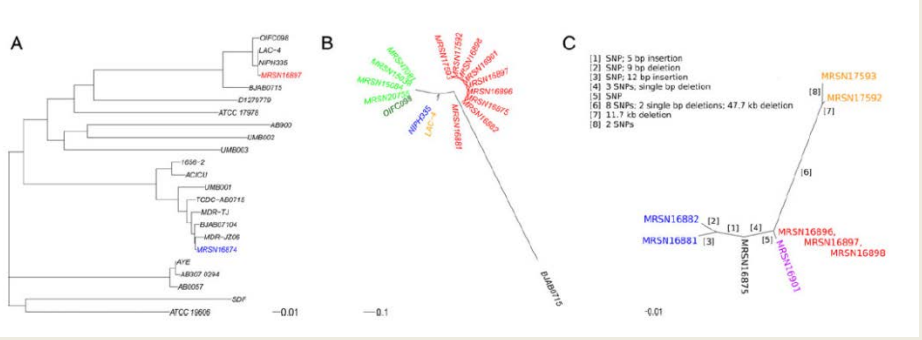
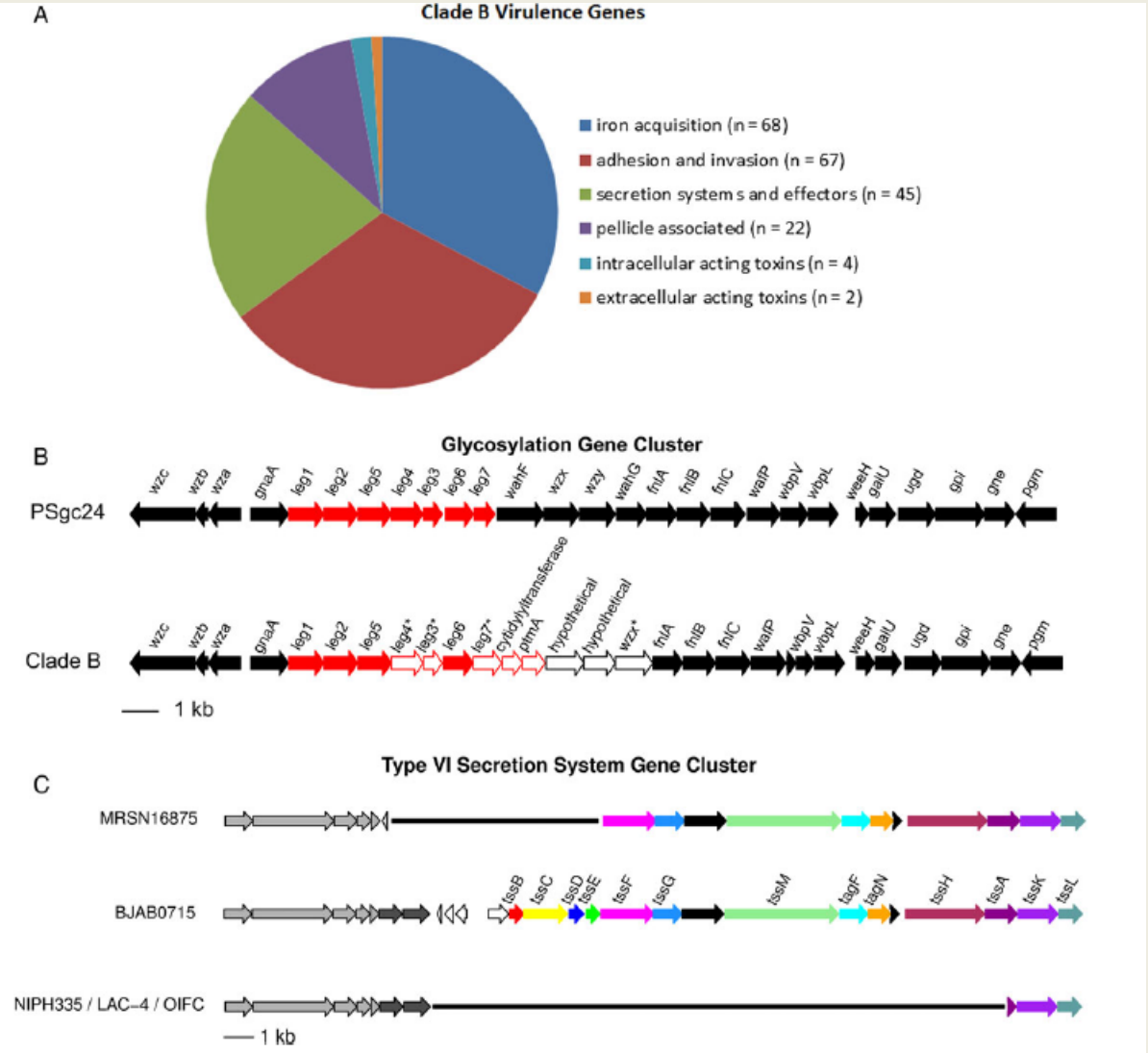
Fatal Outbreak of an Emerging Clone of Extensively Drug-Resistant *Acinetobacter baumannii* With Enhanced Virulence

Crystal L. Jones,[1] Megan Clancy,[2] Cary Honnold,[3] Shweta Singh,[1] Erik Snesrud,[4] Fatma Onmus-Leone,[4] Patrick McGann,[4] Ana C. Ong,[4] Yoon Kwak,[4] Paige Waterman,[4] Daniel V. Zurawski,[1] Robert J. Clifford,[4] and Emil Lesho[4]

[1]Department of Wound Infections, Walter Reed Army Institute of Research, Silver Spring, Maryland; [2]Providence Alaska Medical Center, Anchorage; [3]Department of Pathology, and [4]Multidrug-Resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, Silver Spring, Maryland





- **MLST 10 -related to strains from the Czech Republic, CA, and Germany in 1994, 1997, and 2003, respectively.**
- **In murine models, clade B more virulent > highly virulent reference strain AB5075.**
- **Fe metabolism, protein secretion, and glycosylation.**
- **Developed a PCR assay**

RESEARCH ARTICLE

Joint Transcriptional Control of Virulence and Resistance to Antibiotic and Environmental Stress in *Acinetobacter baumannii*

Michael J. Gebhardt,[a] Larry A. Gallagher,[b] Rachael K. Jacobson,[a] Elena A. Usacheva,[c] Lance R. Peterson,[c] Daniel V. Zurawski,[d] Howard A. Shuman[a]

300 genes were required for survival and/or growth of *A. baumannii* inside *G. mellonella* larvae; established virulence factors and several novel genes.

Among these were more than 30 transcription factors required for growth in *G. mellonella*.

A subset of the transcription factors was also found to be required for resistance to antibiotics and environmental stress (efflux pumps transcriptional regulators, aminoglycoside phosphoyransferase)

Conclusion and Questions??

- **Resistance an virulence are being linked; systems biology approach may reveal a tight association**
- **Many genes involved in resistance may have dual roles? (OMVs, β-Lactamase different than others; OMP A, LpxC)**
- **Is the linking of resistance and modification of virulence a strategy to prolong colonization?**

Acinetobacter