Acinetobacter to target organ: Is biofilm the missing link?

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INTRODUCTION

Resources for Genetic and Genomic Analysis of Emerging Pathogen *Acinetobacter baumannii*

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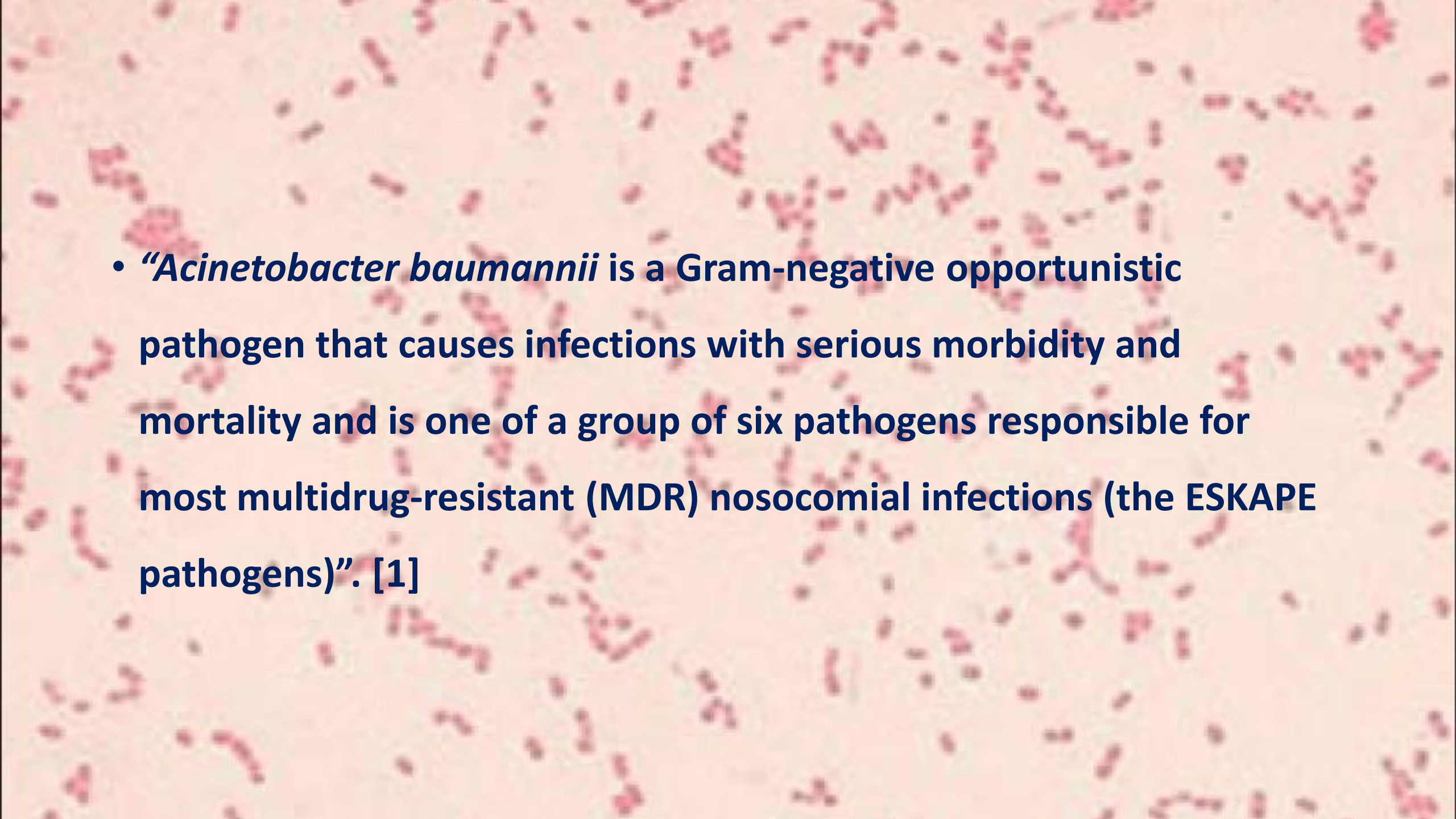
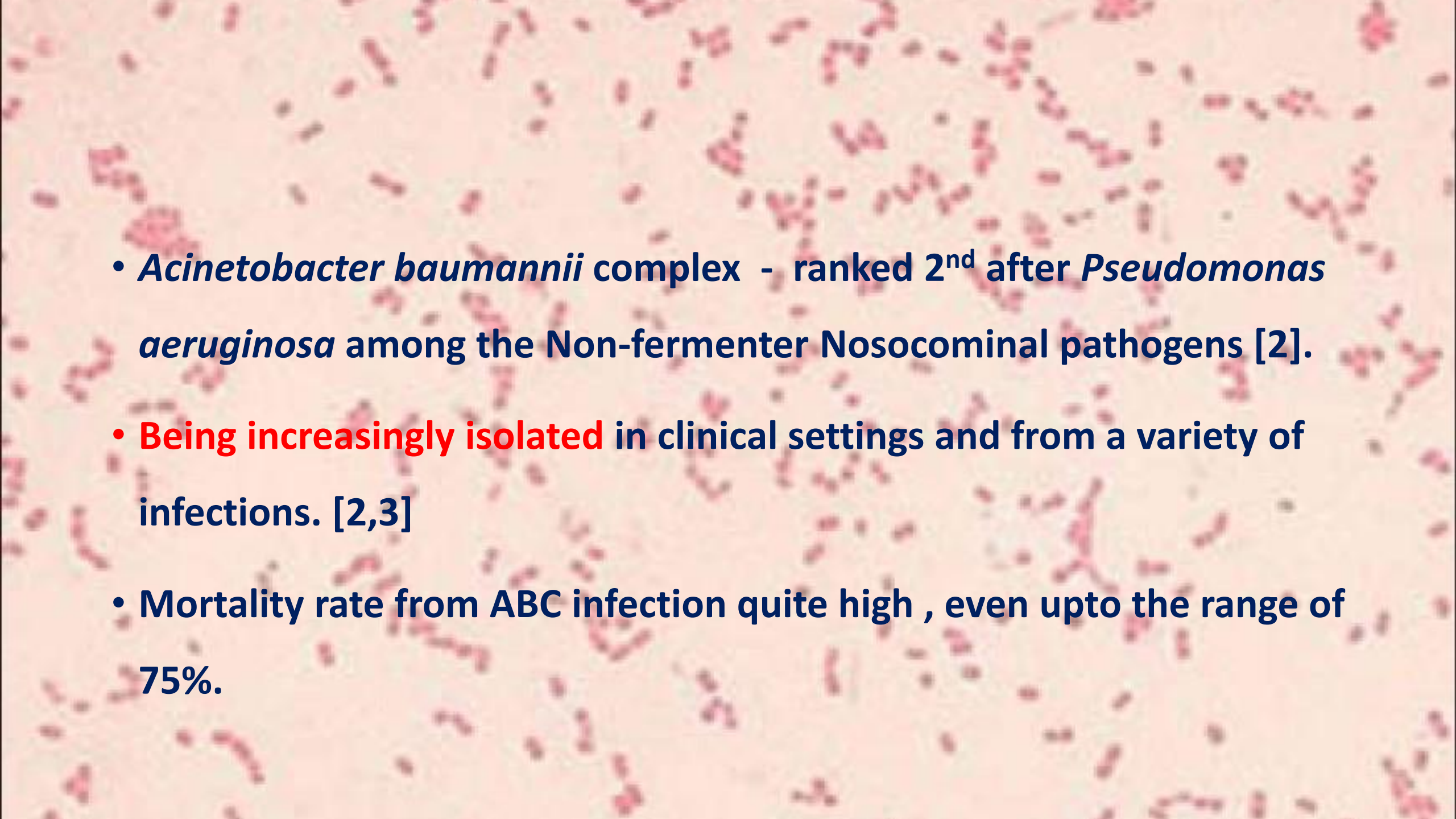
Research

Carbapenem-Nonsusceptible *Acinetobacter baumannii*, 8 US Metropolitan Areas, 2012–2015



- *"Acinetobacter baumannii* is a Gram-negative opportunistic pathogen that causes infections with serious morbidity and mortality and is one of a group of six pathogens responsible for most multidrug-resistant (MDR) nosocomial infections (the ESKAPE pathogens)". [1]



- ***Acinetobacter baumannii*** **complex - ranked 2nd after** ***Pseudomonas aeruginosa*** **among the Non-fermenter Nosocominal pathogens [2].**
- **Being increasingly isolated in clinical settings and from a variety of infections. [2,3]**
- **Mortality rate from ABC infection quite high , even upto the range of 75%.**

- There is a continuing spread of Carbapenem resistant *Acinetobacter baumannii*
- Spread by few clonal lineages
- ICL 2 is most prevalent worldwide and spreading (Acinetobacter 2017. 11th International Symposium on the Biology of Acinetobacter. Sevilla, Spain). [4]

EUROPE TO USA

- **European clones EU I, II, III**
- **Renamed WW 1, 2, 3**
- **CRAB (Carbapenem resistant *A baumannii*) [5]**

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Epidemiology

Molecular Epidemiology of Carbapenem-Nonsusceptible *Acinetobacter baumannii* in the United States

Jennifer M. Adams-Haduch, Ezenwa O. Onuoha, Tatiana Bogdanovich, Guo-Bao Tian, Jonas Marschall, Carl M. Urban, Brad J. Spellberg, Diane Rhee, Diane C. Halstead, Anthony W. Pasculle, Yohei Doi

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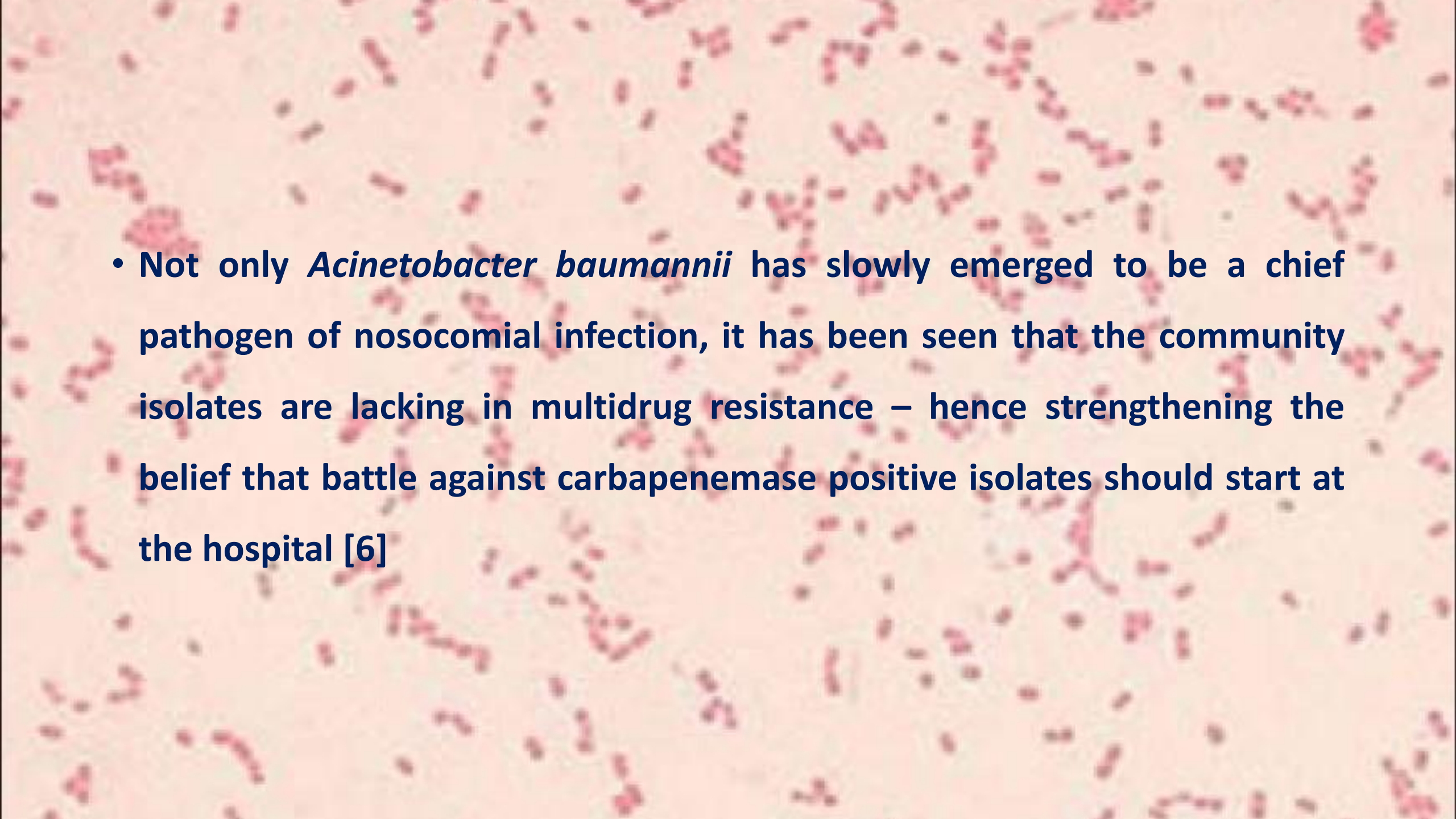
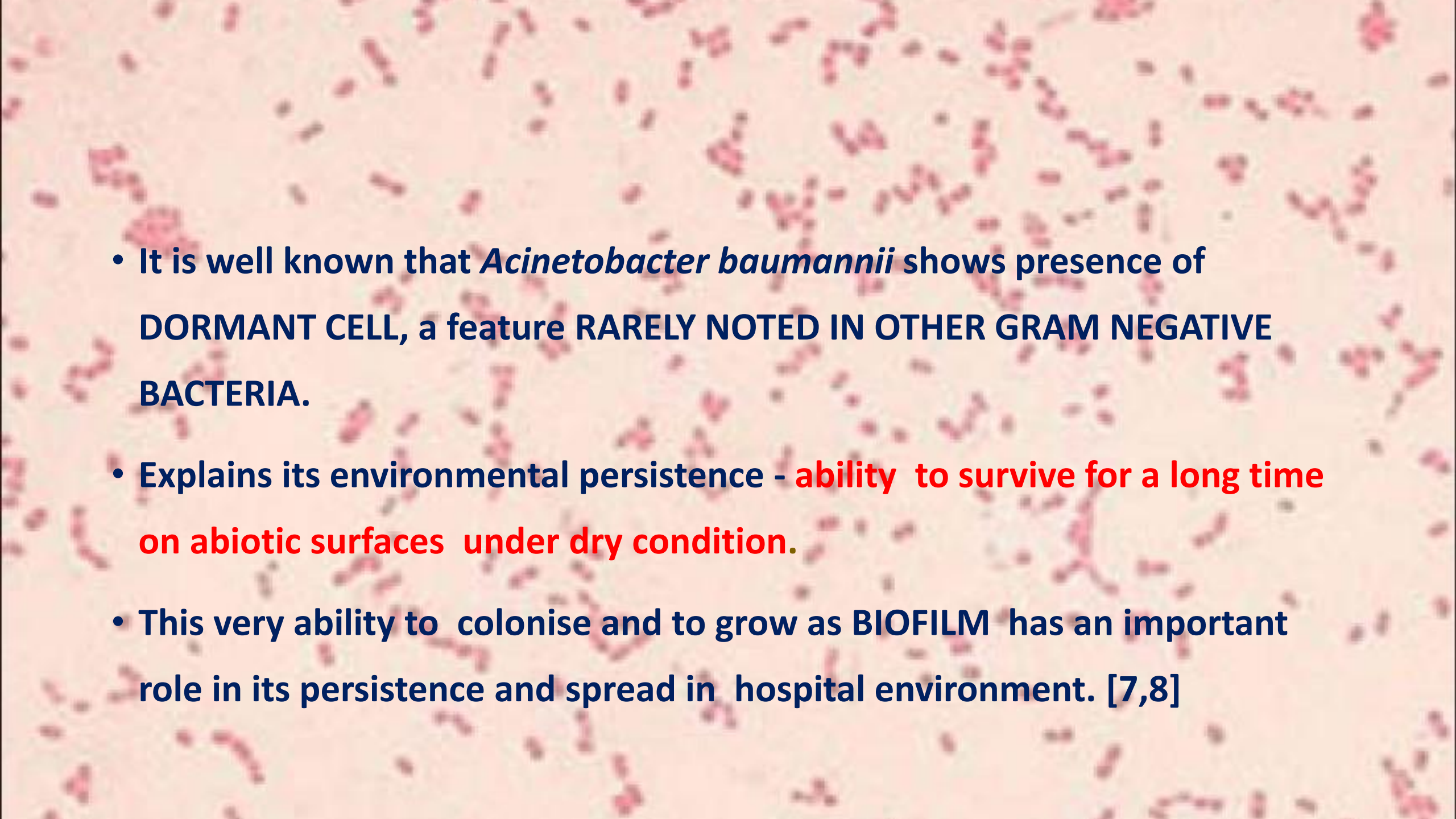
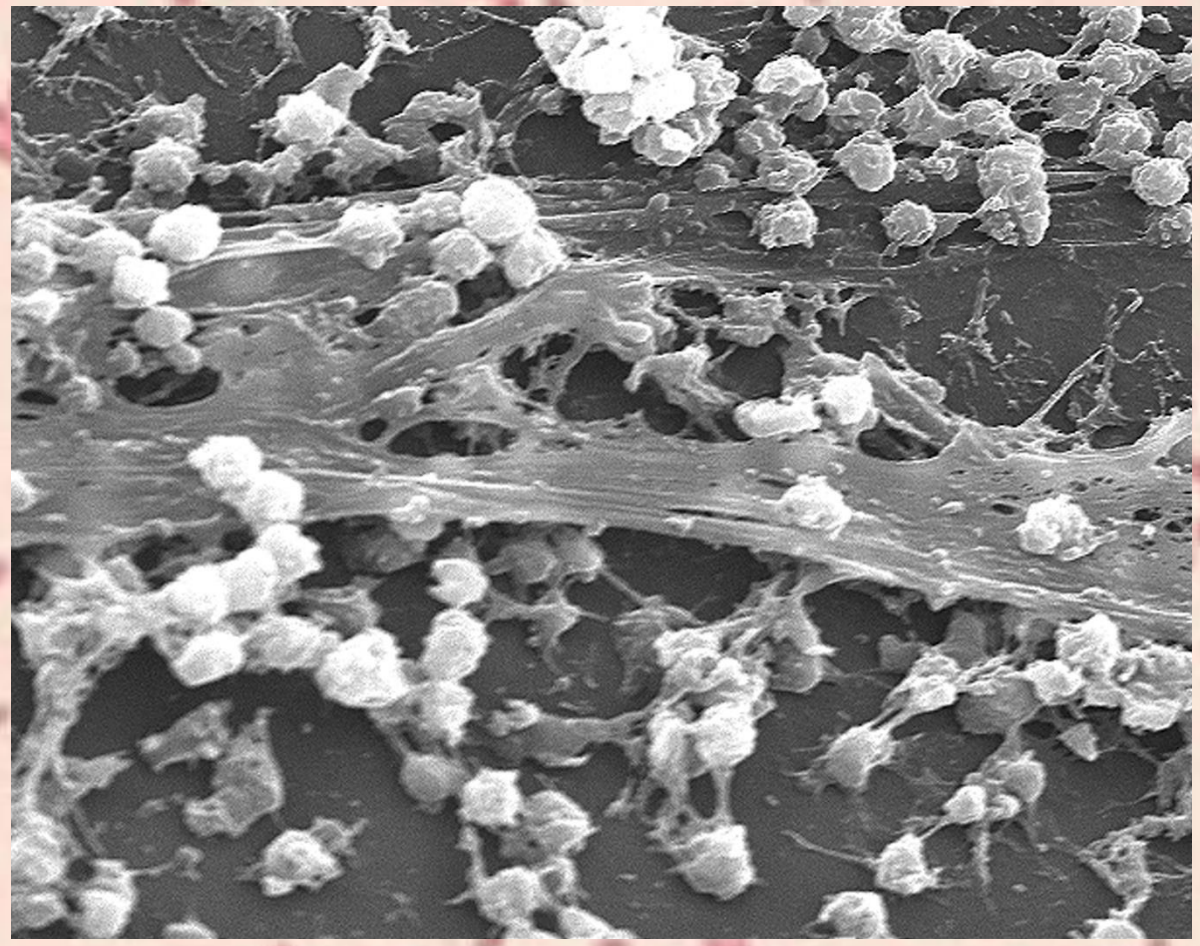


- Not only *Acinetobacter baumannii* has slowly emerged to be a chief pathogen of nosocomial infection, it has been seen that the community isolates are lacking in multidrug resistance – hence strengthening the belief that battle against carbapenemase positive isolates should start at the hospital [6]



- It is well known that *Acinetobacter baumannii* shows presence of DORMANT CELL, a feature RARELY NOTED IN OTHER GRAM NEGATIVE BACTERIA.
- Explains its environmental persistence - ability to survive for a long time on abiotic surfaces under dry condition.
- This very ability to colonise and to grow as BIOFILM has an important role in its persistence and spread in hospital environment. [7,8]

“Biofilm is a microbially derived sessile community characterised by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced , and exhibit an altered phenotype with respect to growth rate and gene transcription”. [9]



RELATIONSHIP BETWEEN BIOFILM FORMATION AND DISEASE.

- **The mechanisms –**

  (a) Detachment of cells or cell aggregates for medical device biofilm, resulting in blood stream and urinary tract infection.

  (b) Production of endotoxin.

  (c) Resistance to Host immune system.

  (d) Provision of a niche for generation of drug resistance [9]

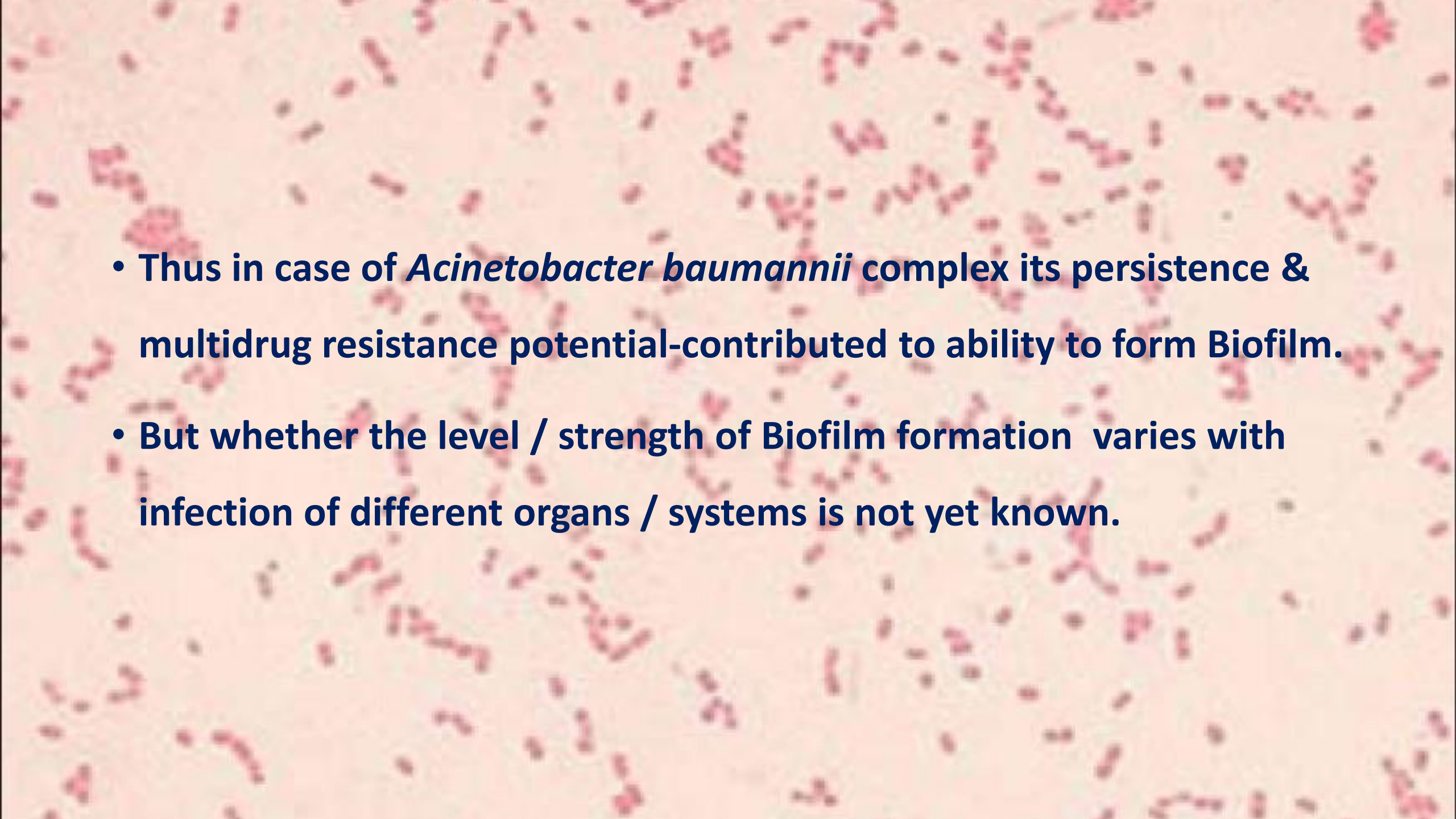
BIOFILM CONFERS AND INHERENT RESISTANCE TO ANTIBIOTIC AS WELL.

- **The mechanisms -**

  (a) Delayed penetration of antibiotic through biofilm

  (b) Altered growth rate

  (c) Other physiological changes due to biofilm. [9]



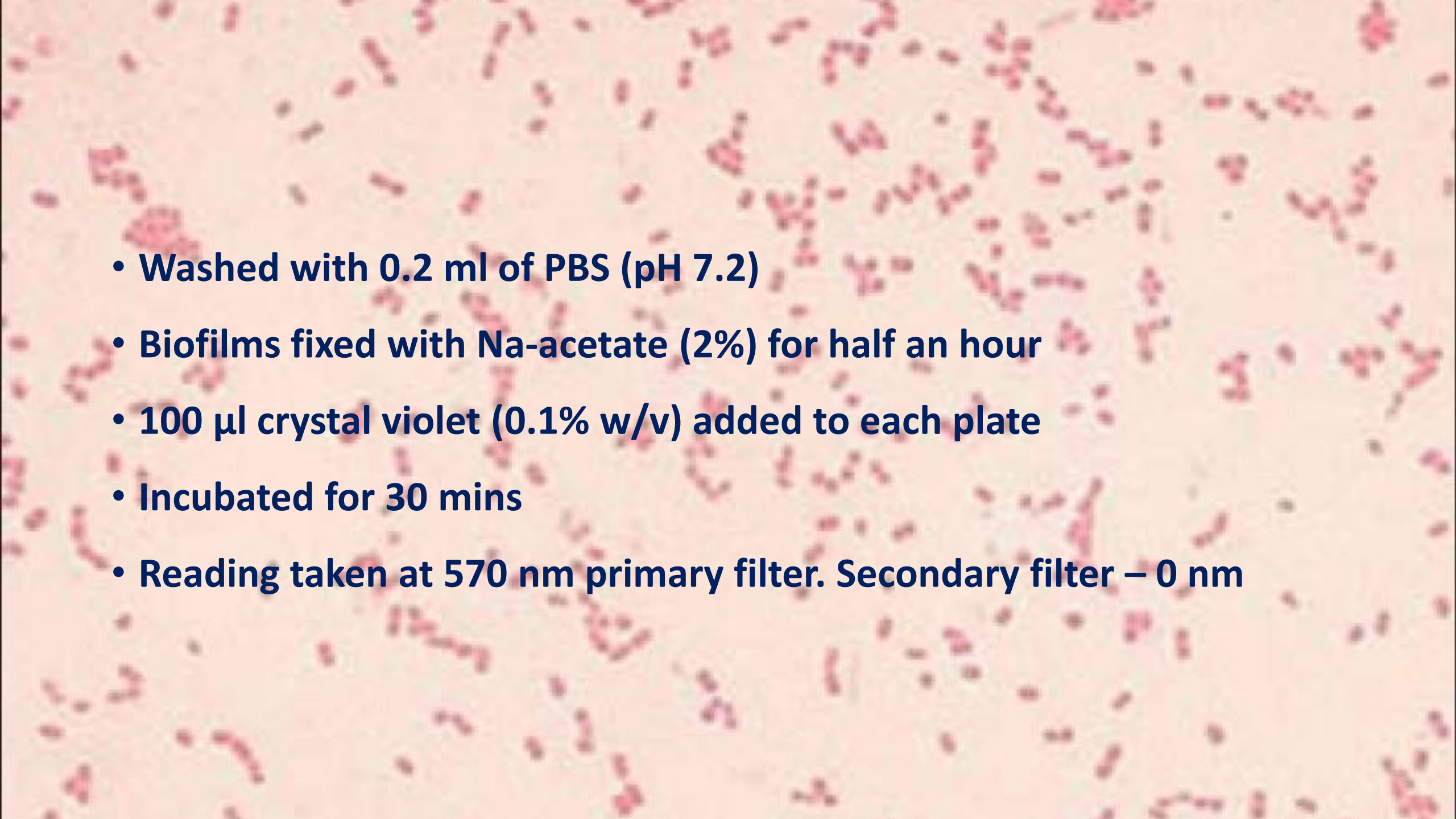
- Thus in case of *Acinetobacter baumannii* complex its persistence & multidrug resistance potential-contributed to ability to form Biofilm.
- But whether the level / strength of Biofilm formation varies with infection of different organs / systems is not yet known.

- THE PRESENT STUDY WAS AIMED AT FINDING OUT ANY POSSIBLE ASSOCIATION BETWEEN BIOFILM FORMING CAPACITY AND ORGANOTROPISM.


MATERIALS
AND
METHODS

- Total 136 isolates of *Acinetobacter baumannii* from different samples
- Samples included – sputum, ET Tube, pus, urine, blood, central venous catheter and cerebrospinal fluid.
- Strength of Biofilm formation – Tissue culture plate method [10,11,12,13,14]

- Each isolate - subcultured on Mac Conkey agar.
- Two colonies of *A baumannii* were inoculated in 5ml of BHI_{SUC}
- Incubated for 18 hrs (to bring to stationery phase)
- Diluted 1 in 100 v/v in same broth
- Poured 200 µl in flat bottomed polysterene plates, covered, incubated at 37^0 c for 24 hrs
- Contents gently tapped to remove



- Washed with 0.2 ml of PBS (pH 7.2)
- Biofilms fixed with Na-acetate (2%) for half an hour
- 100 µl crystal violet (0.1% w/v) added to each plate
- Incubated for 30 mins
- Reading taken at 570 nm primary filter. Secondary filter – 0 nm

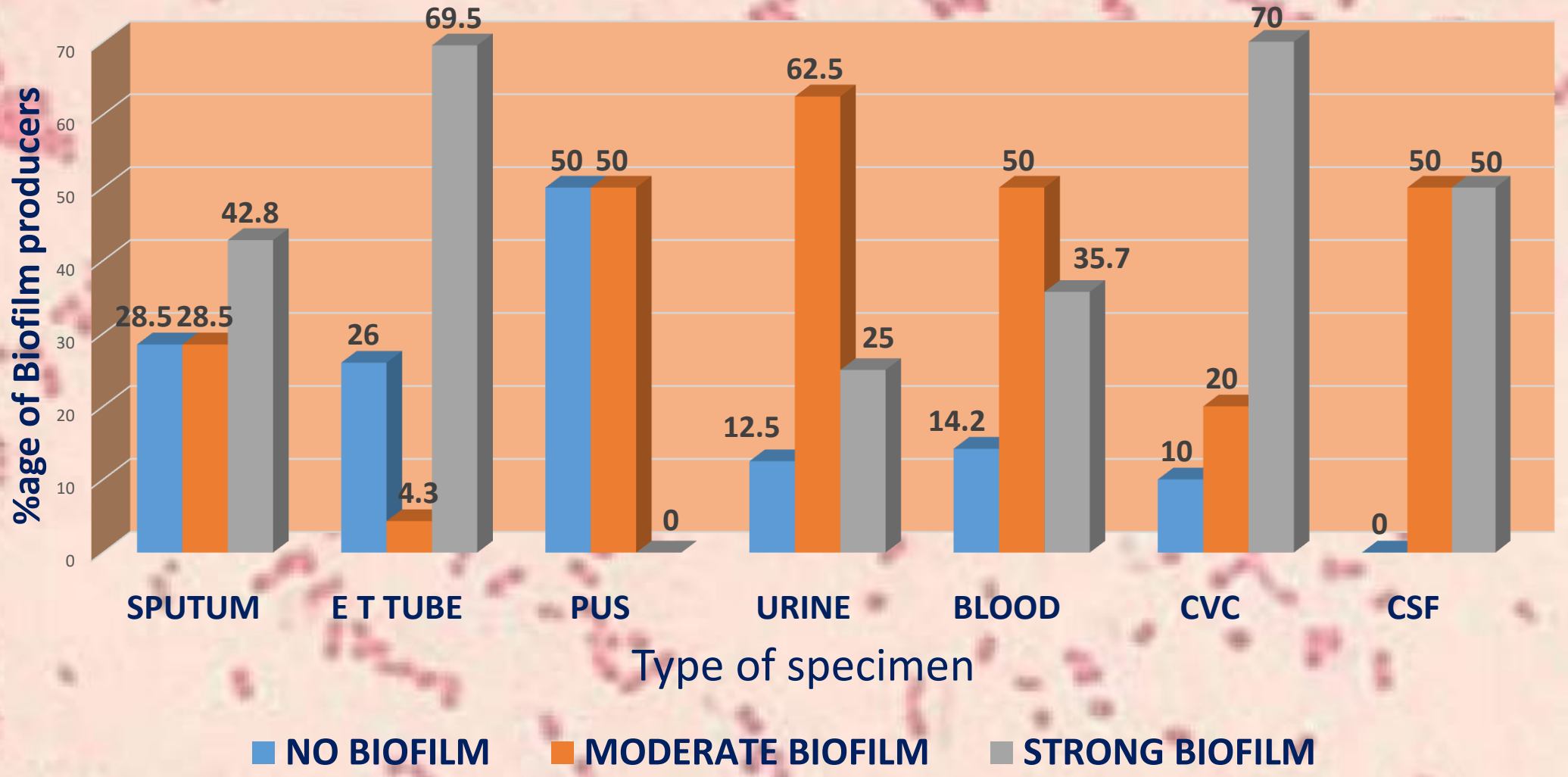
- Strengths were graded as per Christensen et al. [13,14]
- OD value < 0.120 – No Biofilm
- OD value 0.120 – 0.240 - Moderate Biofilm
- OD value > 0.240 – Strong Biofilm


RESULTS

TABLE 1: Association between biofilm strength and infection of different sites by *A baumannii*. (The figures in parenthesis indicate percentage).

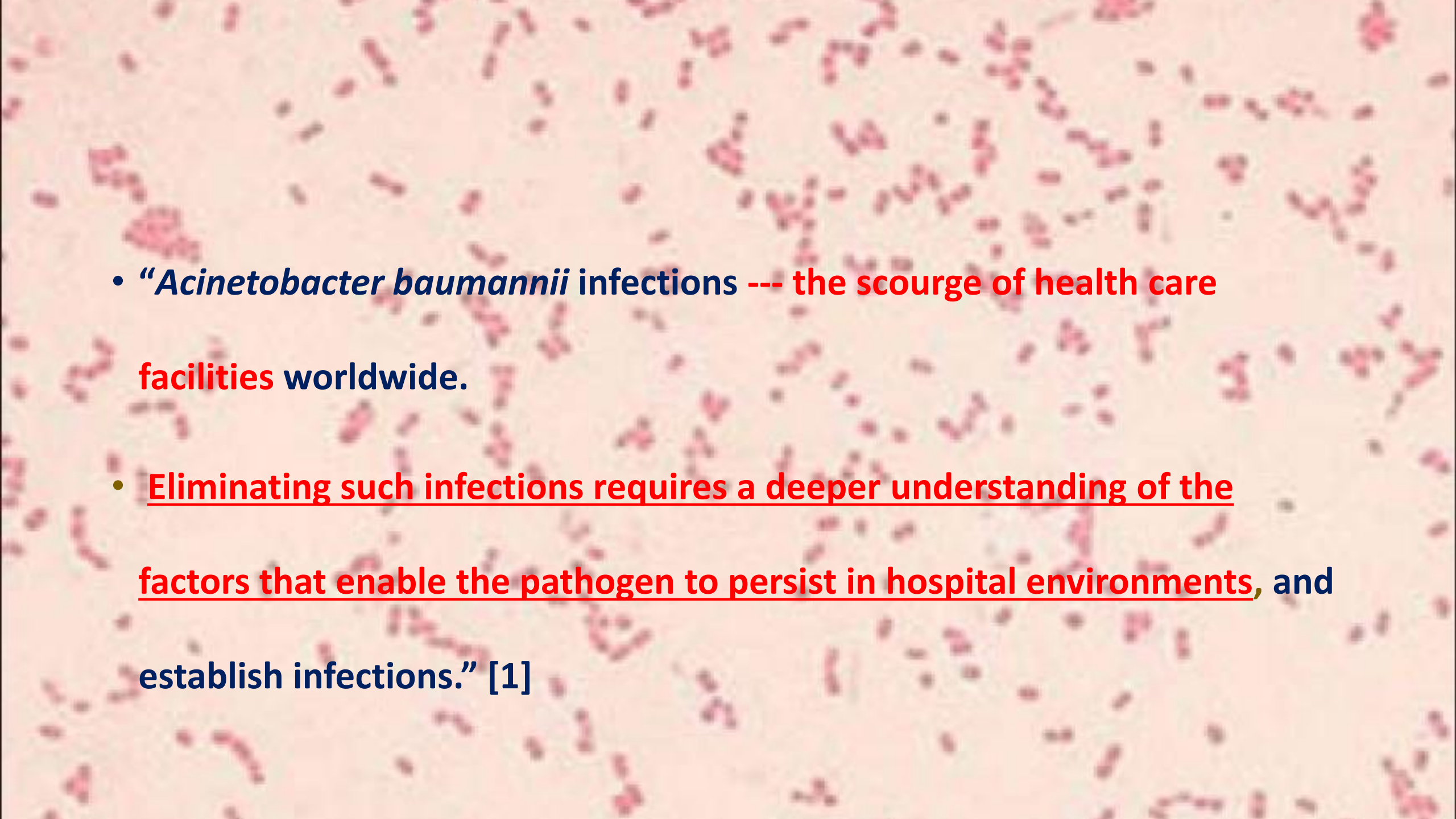
TYPES OF SAMPLES	NO BIOFILM	MODERATE BIOFILM	STRONG BIOFILM	TOTAL
SPUTUM	4 (28.5)	4 (28.5)	6 (42.8)	14
E T TUBE	12 (26.0)	2 (4.3)	32 (**69.5**)	46
PUS	4 (50.0)	4 (50.0)	-	8
URINE	2 (12.5)	10 (62.5)	4 (25.0)	16
BLOOD	4 (14.2)	14 (50.0)	10 (35.7)	28
CVC	2 (10.0)	4 (20.0)	14 (**70.0**)	20
CSF	-	2 (50.0)	2 (50.0)	4
TOTAL	28	40	68	136

Figure 1: Association between biofilm strength and infection of different sites by *A baumannii*



- Statistical analysis: Data were analyzed using SPSS (Statistical Package for Social Scientists) version 20.0, IBM, USA. Chi-square test was applied for comparing categorical data at 5% significance level.
- THE DIFFERENCE IN STRENGTH OF BIOFILM FORMATION DEPENDING ON SITES OF INFECTION WAS STATISTICALLY SIGNIFICANT [χ^2 (12) = 41.99. p =0.000]


DISCUSSION



- "*Acinetobacter baumannii* infections --- the scourge of health care facilities worldwide.
- Eliminating such infections requires a deeper understanding of the factors that enable the pathogen to persist in hospital environments, and establish infections." [1]

- **A STRONG ASSOCIATION WAS FOUND OUT BETWEEN STRENGTH OF BIOFILM FORMATION AND ITS VARIATION ACCORDING TO DIFFERENT SITES OF INFECTION.**
- **This study hints that PATHOGENESIS OF BIOFILM FORMATION MAY HAVE A LINK WITH ORGANOTROPISM BY DIFFERENT STRAINS OF *Acinetobacter baumannii* COMPLEX.**

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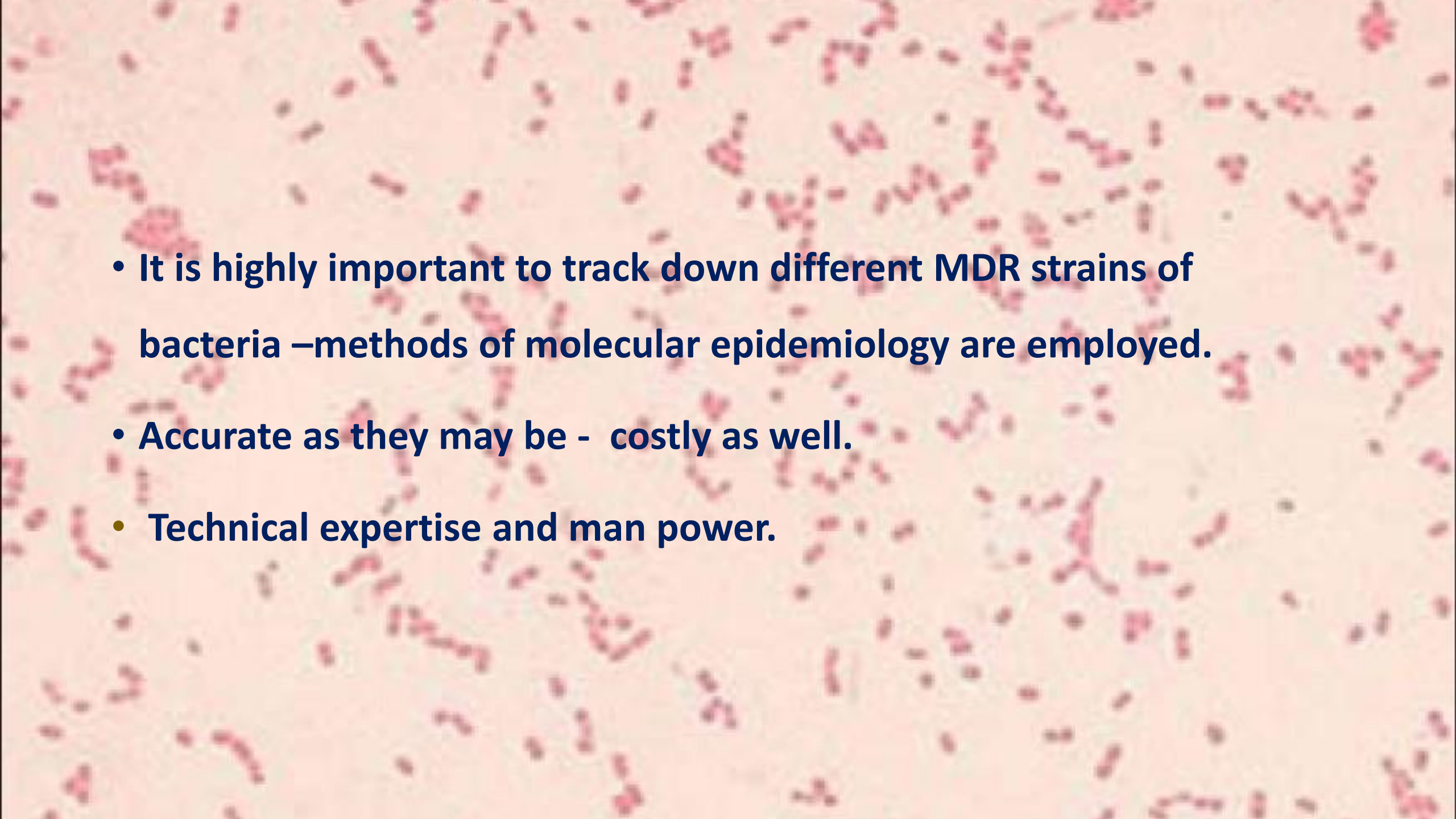
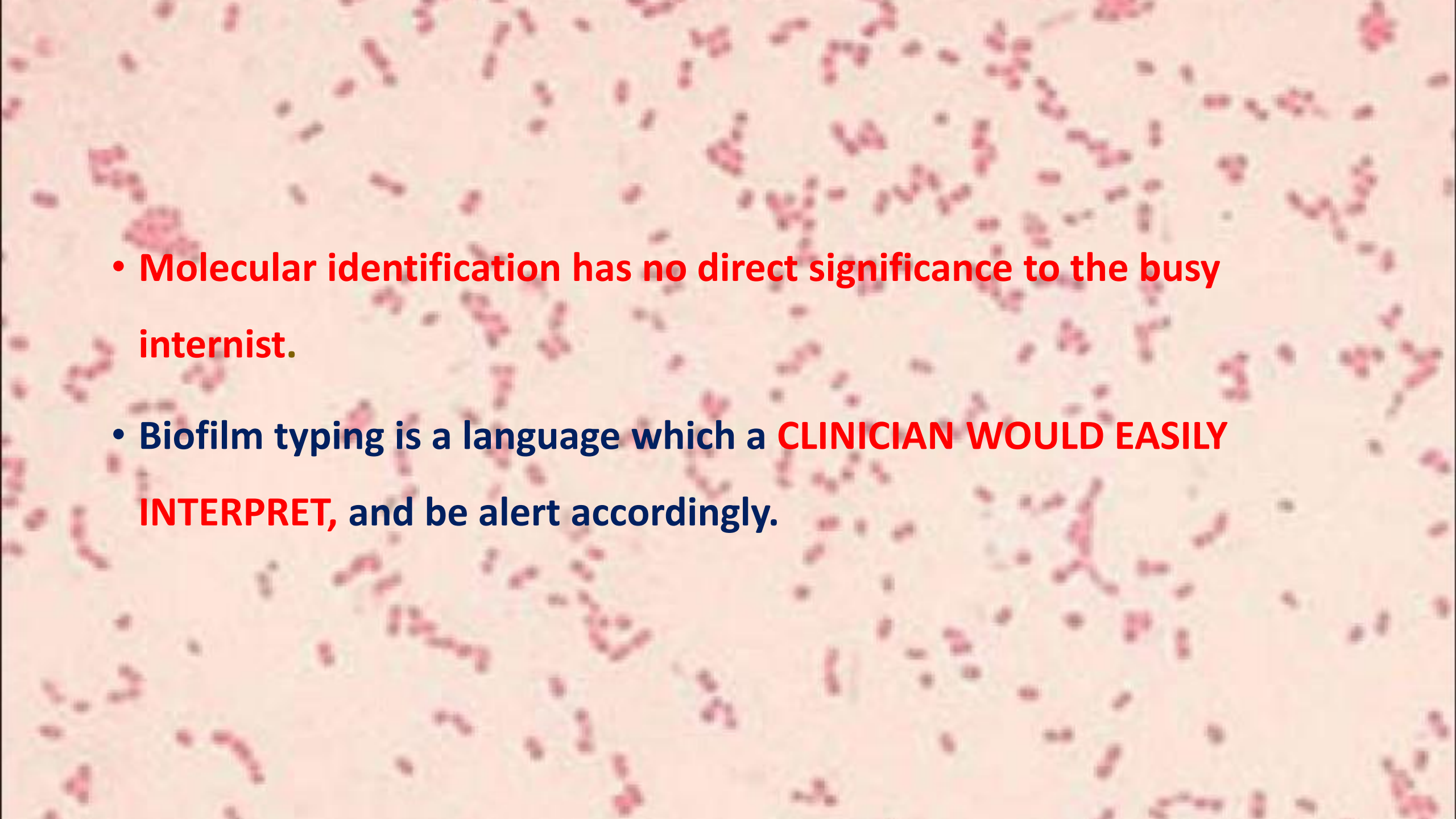


- It is highly important to track down different MDR strains of bacteria –methods of molecular epidemiology are employed.
- Accurate as they may be - costly as well.
- Technical expertise and man power.

- Accepting biofilm as an important virulence factor, and variation of its strength varying with different sites of infection, we suggest **BIOFILM TYPING** by the method described.
- EASY, EFFECTIVE IN A COST RESTRICTED scenario, done with SIMPLE INSTRUMENTS like an ELISA reader, AVAILABLE almost in every laboratory set up.



- Molecular identification has no direct significance to the busy internist.
- Biofilm typing is a language which a CLINICIAN WOULD EASILY INTERPRET, and be alert accordingly.


Thank you