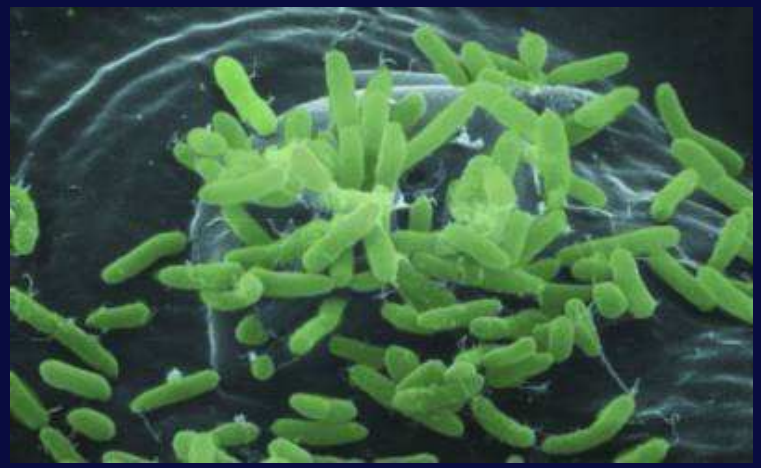
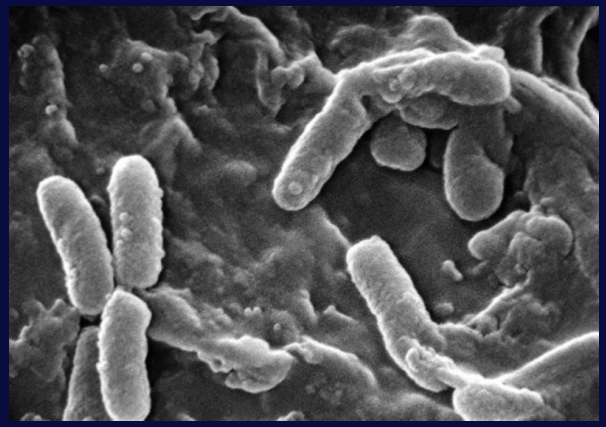
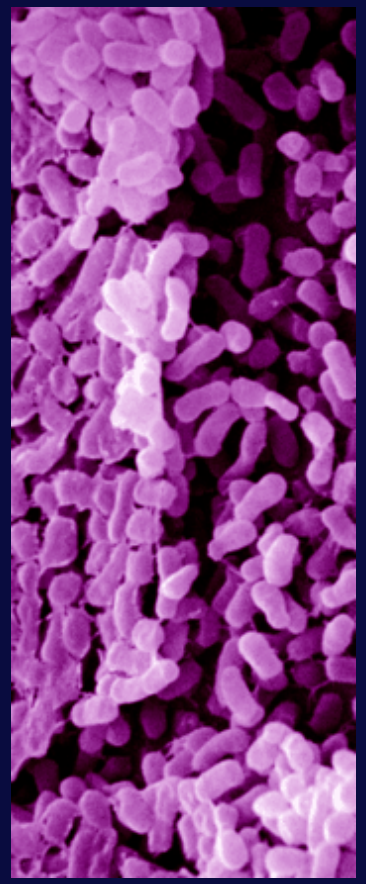
EPIDEMIOLOGY AND CONTROL OF INFECTIONS CAUSED BY NON-FERMENTING GRAM-NEGATIVE RODS







GENERAL CHARACTERISTICS

-MOTILE, NON-SPORULATED RODS

- UTILIZE CARBOHYDRATES THROUGH AEROBIC RESPIRATION AND SOMETIMES VIA UNUSUAL METABOLIC ROUTES

-ALTHOUGH DESCRIBED AS OBLIGATE AEROBES, THEY CAN GROW ANAEROBICALLY

-SUPERFICIAL GROWTH ON KIA AND TSI MEDIA

- GLUCOSE NON-FERMENTATIVES

LABORATORY DIAGNOSIS AND CULTURE

-FERMENTATION TESTS: KIA AND TSI MEDIA

- PRESENCE OF CYTOCHROME OXIDASE
- CULTURE ON MacConkey AGAR
- DETECTION OF GLUCOSE FERMENTATION
- PIGMENT PRODUCTION: PYOVERDIN AND PYOCYANINA (FLO AND TECH AGAR)
- UREA HYDROLISIS: CHRISTENSEN AGAR
- DESNITRIPHICATION TESTS
- INDOLE PRODUCTION
- DECARBOXILATION OF AMINOACIDS
- HYDROLISIS OF SCULINE
- STAIN PROCEDURES OF FLAGELLA, PILI…..

TAXONOMIC CLASSIFICATION

-EXTENSIVE RANGE OF GENERA AND SPECIES

-RECENT COMPREHENSIVE ANALYSES OF THE GROUP HAVE LED TO REVISED CLASSIFICATIONS; MANY SPECIES HAVE BEEN ALLOCATED TO NEW GENERA

-15 FAMILIES:(*Alacaligenaceae, Alteromonadaceae, Brucellaceae, Burkholderiaceae, Caulobacteraceae, Comamonadaceae, Flavobacteriaceae, Methylobacteriaceae, Moraxellaceae, Oceanospirillaceae, Pseudomonadaceae, Rhizobiaceae, Sphingomonodaceae y Xanthomonadaceae*)

- STANDARD DESIGNATION: CDC (*CENTERS FOR DISEASE CONTROL AND PREVENTION*)

RELEVANT GRAM-NEGATIVE NON-FERMENTERS

Pseudomonas aeruginosa

Acinetobacter baumannii, A. lwoffii

Stenotrophomonas maltophilia

Burkholderia cepacia, B. mallei & B. pseudomallei

Moraxella catarrhalis

CULTURE AND IDENTIFICATION

FACTORS DIFFICULTING IDENTIFICATION:

- SOME SPECIES ARE VERY RARE
- LABORATORY STAFF WITH POOR KNOWLEDGE ABOUT THE MANAGEMENT OF THESE ISOLATES
- NEED OF SPECIFIC CULTURE MEDIA
- SLOW GROWTH RATE IN SOME SPECIES
- LACK OF CONTROL ON MEDIA QUALITY
- LOW RATES OF CORRECT IDENTIFICATION WHEN USING COMMERCIAL EQUIPMENT

USEFUL TIPS FOR THE IDENTIFICATION

1.- INCREASING RATE OF ISOLATION FROM IMMUNOCOMPROMISED PATIENTES: SUFFERING FROM METABOLIC DISEASES, CANCER, WOUNDS, UNDER CORTICOSTEROIDS OR ANTIBIOTIC TREATMENT

2.- MOST ARE SAPROPHYTES FOUND IN SOIL, WATER AND OTHER ENVIRONMENTS (MANY OF THEM ARE ORIGIN OF HUMAN INFECTIONS AS WATER BATHS, DISINFECTANTS, HAND MOISTURES, MEDICAL EQUIPMENT, SKIN….)

USEFUL TIPS FOR THE IDENTIFICATION

3.- THEY CAN CAUSE SPECIFIC INFECTIONS (SEPTICAEMIA, PNEUMONIA, URINARY TRACT INFECTION…)

4.- MOST OF THEM GROW AS SMALL COLONIES ON BLOOD AGAR, POOR GROWTH ON MacConkey , KIA / TSI-NEGATIVE AND OXIDASE-POSITIVE.

5.- MULTIRESISTANT TO MANY ANTIBIOTICS

MOST FREQUENTLY ISOLATED SPECIES

1. *Pseudomonas aeruginosa:*
   - large colonies, grape-like odor
   - green pigmentation
   - oxidase-positive

2. *Acinetobacter baumannii:*
   - pinkish colonies on MacConkey agar
   - oxidase-negative

3. *Stenotrophomonas maltophilia:*
   - good rate of growth on blood and MacConkey agar
   - oxidase-negative
   - some strains produce yellow pigmentation

COMMERCIAL EQUIPMENT

API 20E

BBL Crystal Enteric/Nonfermenter

BD PHOENIX

REMAL RapID ONE

Biolog GN Microplate

MicroScan

VITEK 2

CHOOSING A COMMERCIAL EQUIPMENT

- AFTER EVALALUATION OF ACCURACY, COST-EFECTIVENESS, EFFECTS OVER WORK-FLOW

-GOOD RESULTS FOR *Pseudomonas aeruginosa* AND FOR SOME SPECIES OF *Acinetobacter* & *Stenotrophomonas maltophilia*

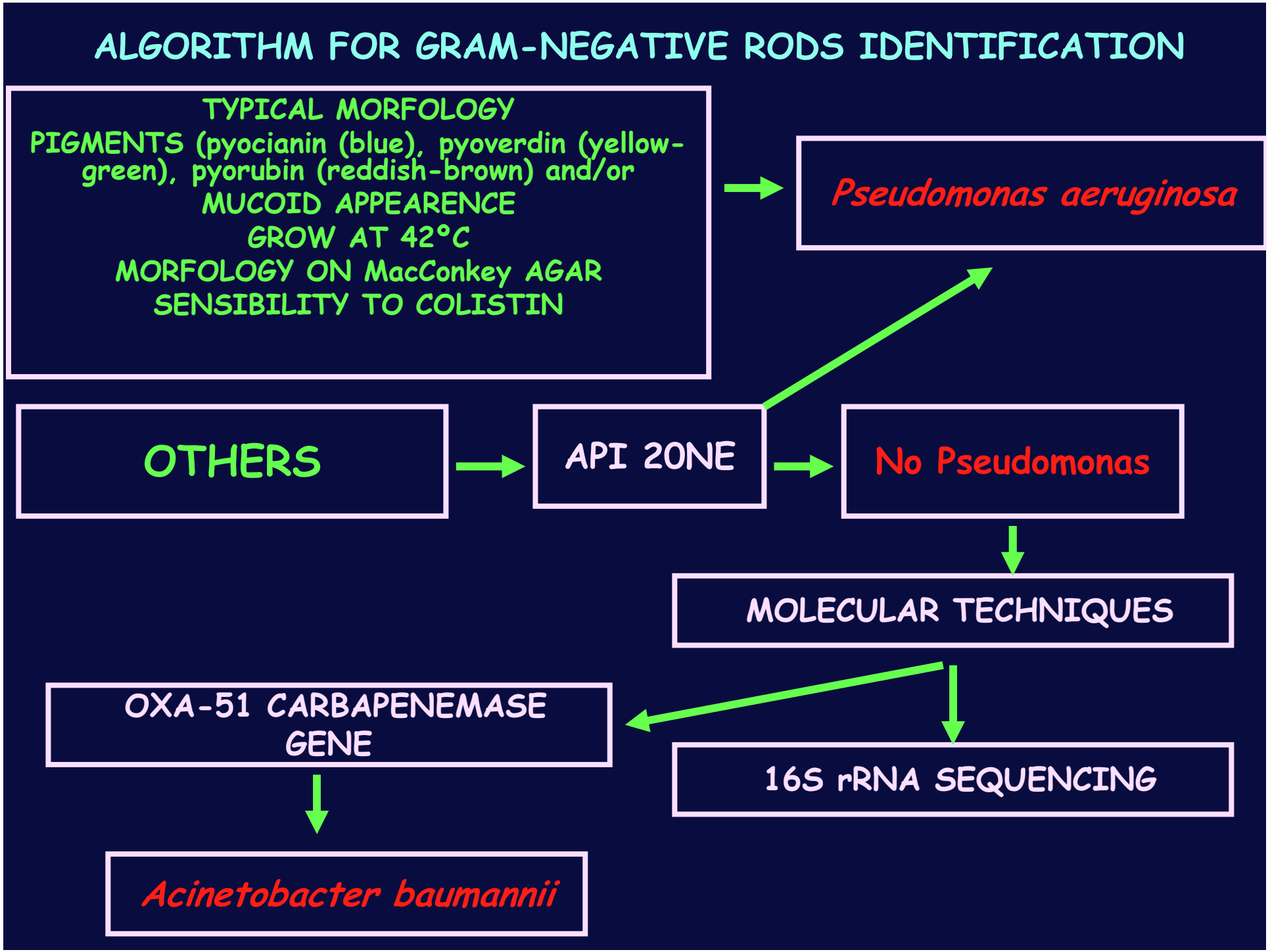
- DEFINITIVE IDENTIFICATION: CONVENTIONAL PROCEDURES

MOLECULAR TECHNIQUES USED FOR DIAGNOSIS

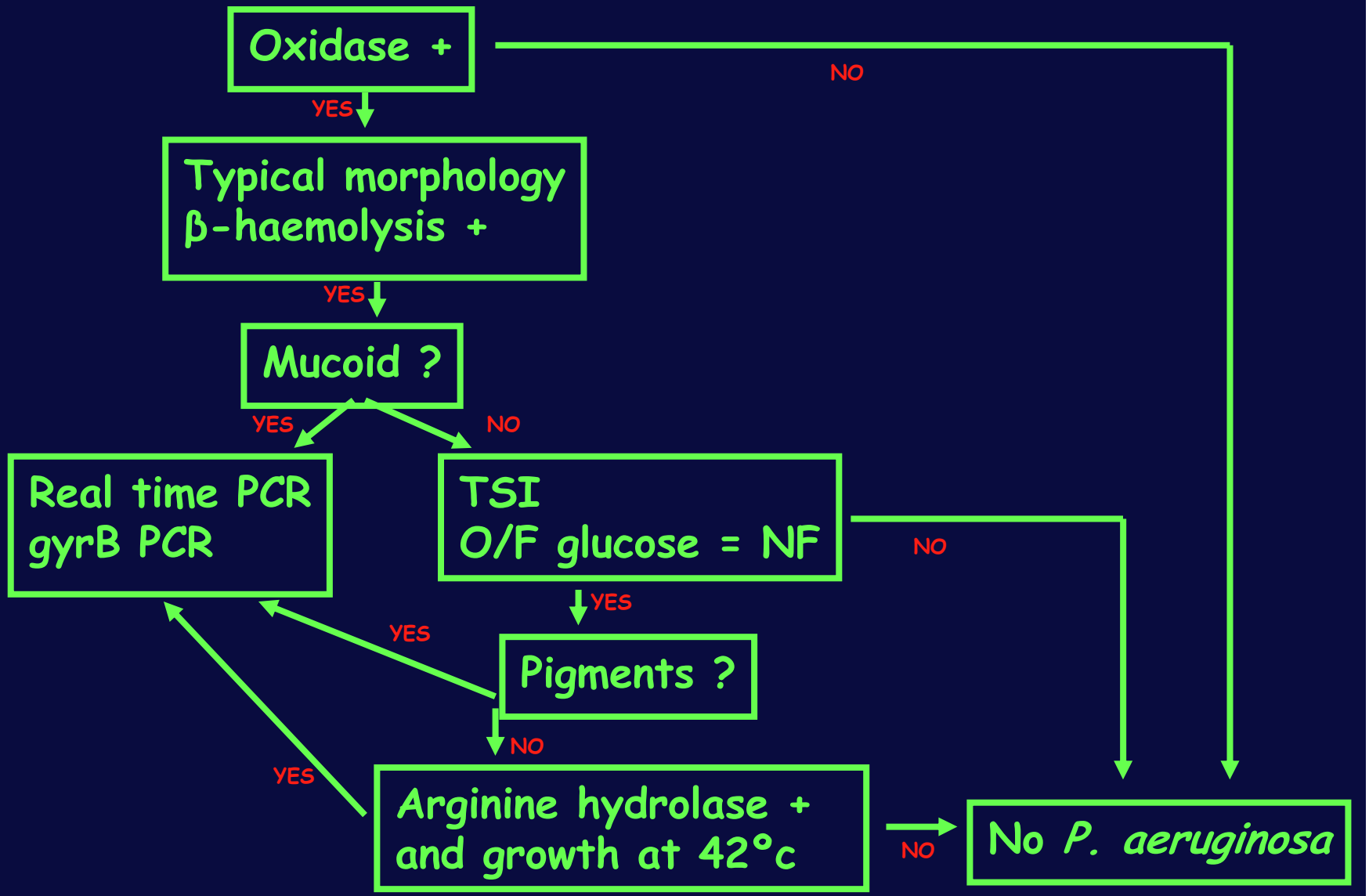
1. rRNA 16S GENE SEQUENCING
2. REAL-TIME PCR
3. FLUORENCENCE IN SITU HYRIDIZATION (FISH)

ADVANTAGES:

- USEFUL FOR IDENTIFICATION OF RARE SPECIES
- WHEN CONVENTIONAL TECHNIQUES DO NOT SUCCEED-
- WHEN RAPID RESULTS ARE NEEDED (FROM 90 MINUTES)
- FISH SYSTEM IS LOW COST AND DO NOT REQUIRE HIGH-LEVEL TECHNOLOGY


ALGORITHM FOR GRAM-NEGATIVE RODS IDENTIFICATION
TYPICAL MORFOLOGY
PIGMENTS (pyocianin (blue), pyoverdin (yellow-green), pyorubin (reddish-brown) and/or
MUCOID APPEARENCE
GROW AT 42°C
MORFOLOGY ON MacConkey AGAR
SENSIBILITY TO COLISTIN
Pseudomonas aeruginosa
OTHERS
API 20NE
No Pseudomonas
MOLECULAR TECHNIQUES
OXA-51 CARBAPENEMASE GENE
16S rRNA SEQUENCING
Acinetobacter baumannii

ALTERNATIVE FLOW CHART FOR IDENTIFICATION



OTHER NON-FERMENTERS

Oxidase +
TSI=K/NC
OF Glucose= non fermenter
No pigments
Negative for (at least one):
- typical morphology
- arginine dihydrolase
- Growth at 42°C
- β-haemolysis

Vitek ID
Rapid NF strip
Real time PCR
gyrB PCR/sequencing
V3 gen sequencing

REAL-TIME PCR

1. *PRIMERS* USED:

   - 16S rDNA

   - *P.aeruginosa gyr*B (gyrPA-398 & gyrPA-620), DNA girasE

   - *P.aeruginosa* ETA (ETA-1 & ETA-2)

   - *P.aeruginosa alg*D (Vic-1 & Vic-2), GDP manose dehydrogenasa)

   - *P.aeruginosa oprl* (OPR-1 & OPR-2), surface lipoprotein

FISH

1. PROBES USED
   - Psae TARGET TO rRNA 23S
   - Psae16S-182 TARGET TO rRNA 16S
2. CHEAPER AND EASIER TO USE THAN REAL-TIME PCR

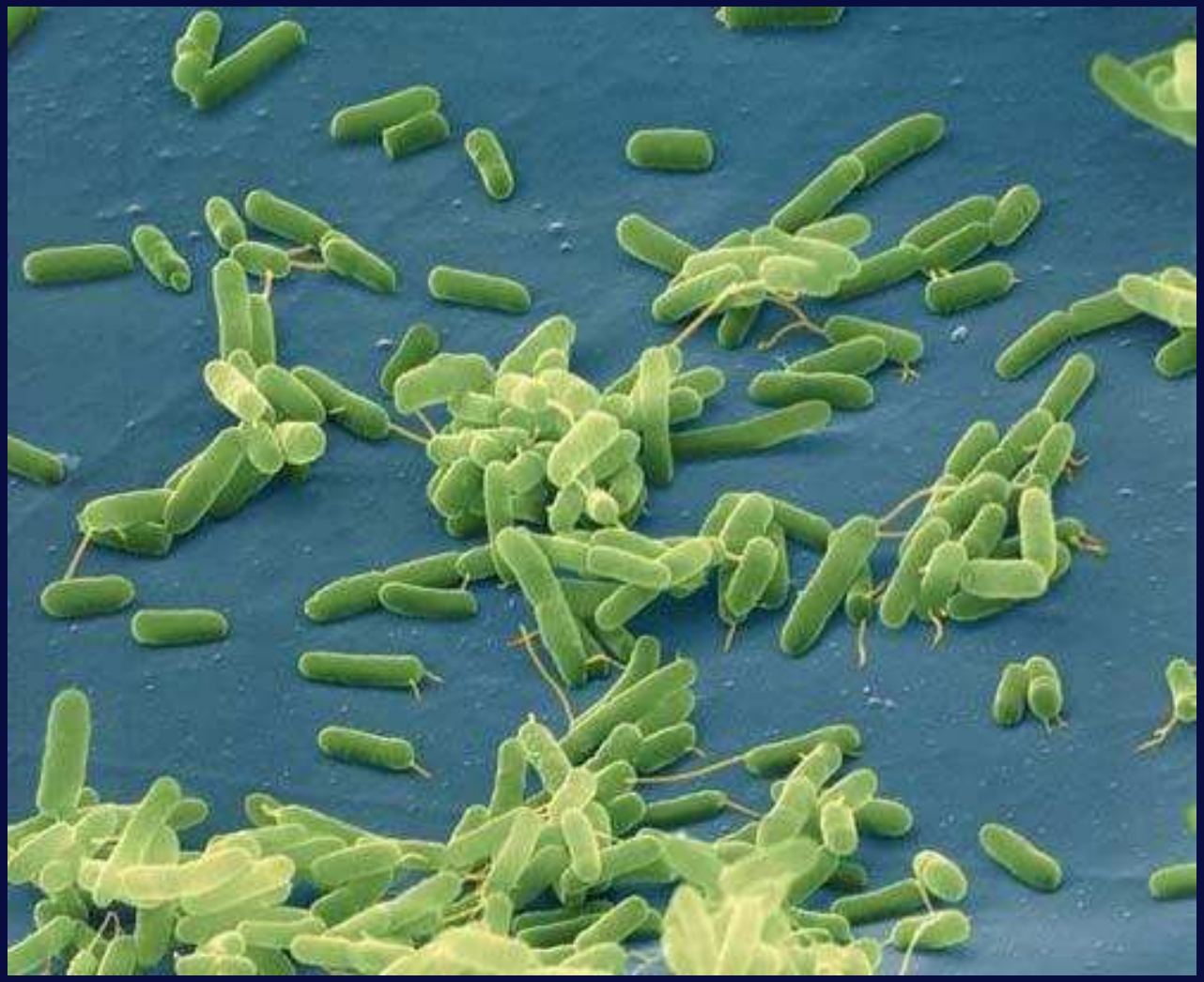
EPIDEMIOLOGY: PATHOGENIC NON-FERMENTERS DATA BASES

1. WHONET
2. SENTRY: SouthAmerica

TOP-TEN NON-FERMENTERS:

1. *P. aeruginosa*
2. *A. baumannii*
3. *S. maltophilia*

Pseudomonas aeruginosa: EMERGING PATHOGEN IN NOSOCOMIAL INFECTIONS



Pseudomonas aeruginosa:
DESCRIPTION

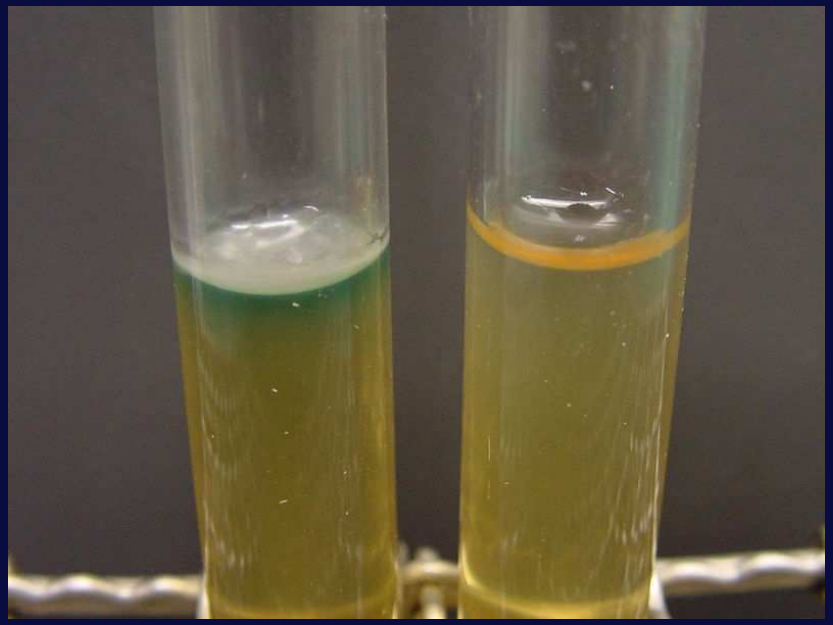
- GRAMNEGATIVE ROD
- NON-FERMENTER
- AEROBE
- MOTILE
- CATALASE & OXIDASE PRODUCER

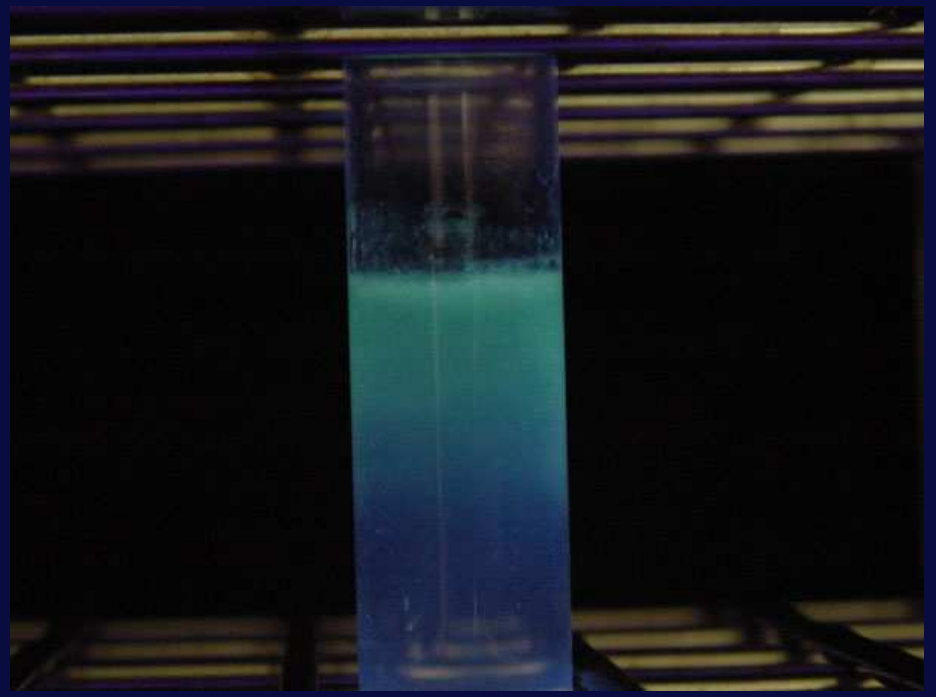
-ISOLATED FROM A WIDE VARIETY OF ENVIRONMENTAL SOURCES

-PIGMENT PRODUCERS (PYOCYANIN, PYOVERDIN & PYORUBIN)

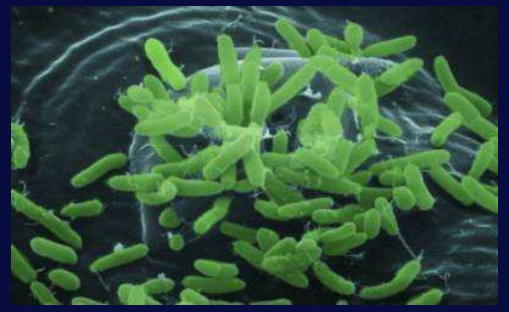
- GROWS ON MOST COMMON CULTURE MEDIA
- GRAPELIKE ODOR

Pseudomonas aeruginosa:
growth in liquid media & pigment production





Pseudomonas aeruginosa:
ECOLOGY

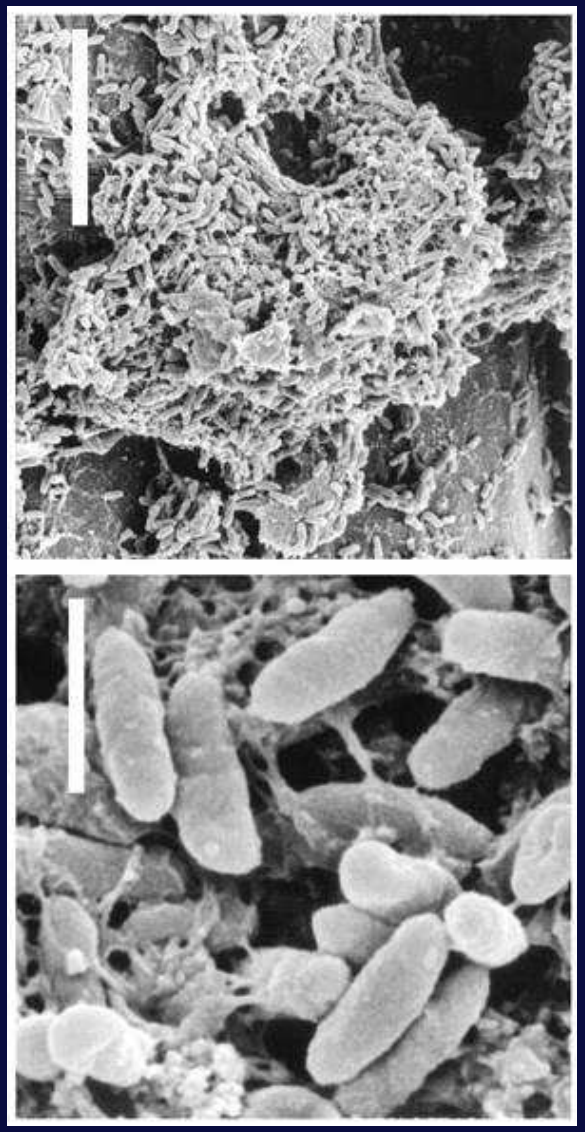


1. NATURAL ENVIRONMENT (UBIQUITOUS)
- WATER, SOIL, VEGETATION, DECAYING ORGANIC MATTER
- SKIN, THROUT, & FAECES OF 2-10% HEALTHY INDIVIDUALS

2. HOSPITAL ENVIRONMENT
- DISINFECTANTS
- SINKS & TAPS
- NON-STERILE LIQUIDS
- DRUGS
- CONTACT LENS SOLUTIONS
- COLONIZATION OF SKIN, GASTROINTESTINAL & RESPIRATORY TRACT OF THE 50% OF PATIENTS UNDER MECHANICAL VENTILATION,PATIENTS TREATED WITH ANTIBIOTICS OR HOSPITALISED FOR EXTENDED PERIODS

Pseudomonas aeruginosa: VIRULENCE FACTORS

1. ADHESINS: FLAGELLA, PILI, LIPOPOLYSACCHARIDE AND ALGINATE (exopolysaccharide that forms a prominent capsule)
2. SECRETED TOXINS AND ENZYMES:
   - EXOTOXIN A (ExoA)
   - PYOCYANIN & PYOVERDIN
   - LasA (serine protease) & LasB (zinc metalloprotease)
   - Alakaline Protease
   - PHOSPHOLIPASE C
   - EXOENZYMES S & T
3. ANTIBIOTIC RESISTANCE



VIRULENCE FACTORS:EFFECTS

-ADHESINS: adherence to host cells for establishing infection; protection from phagocytosis and the activity of antibiotics

-EXOTOXIN A: disrupts protein synthesis by blocking peptide chain elongation in eukaryotic cells; contributes to the dermatonecrosis that occurs in burn wounds, cornel dammage and tissue damage in chronic pulmonary infections; immunosuppressive.

-PYOCYANINE: catalyzes the production of superoxide and hydrogen peroxide, toxic forms of oxygen.

-PYOVERDIN: siderophore that binds iron for use in metabolism

VIRULENCE FACTORS:EFFECTS

-LasA y LasB: act synergistically to degrade elastin; produce lung parenchymal damage and hemorrhagic lesions (ecthyma gangrenosum).

-Alkaline Protease: tissue destruction

-PHOSPHOLIPASE C: heat-labile hemolysin that breaks down lipids and lecithin

-EXOENZYMES S & T: extracellular toxins

-ANTIBIOTIC RESISTANCE: inherently resistant to many antibiotics, it can mutate to even more resistant strains during therapy.

Pseudomonas aeruginosa: INFECTIONS

1. PNEUMONIA
2. CHRONIC PULMONARY INFECTIONS
3. BURN WOUNDS
4. SKIN AND SOFT TISSUES
5. URINARY TRACT
6. BACTEREMIA
7. ENDOCARDITIS

PROBLEMS IN HOSPITALS

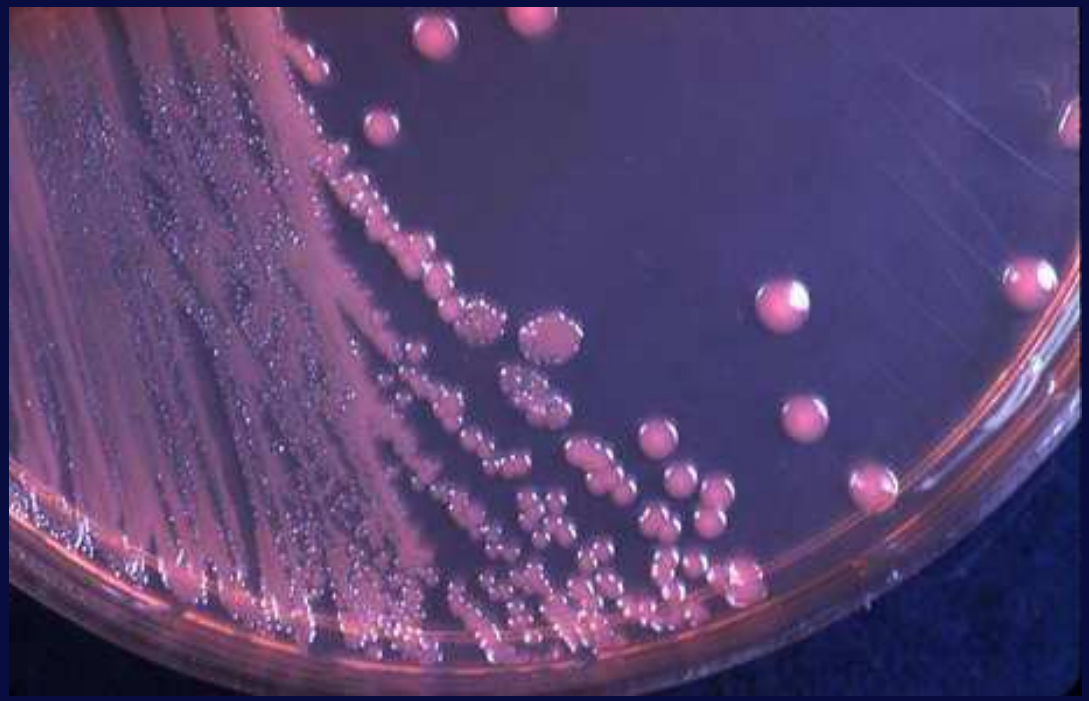
RESISTANCE TO DESECATION AND DISINFECTANT SOLUTIONS

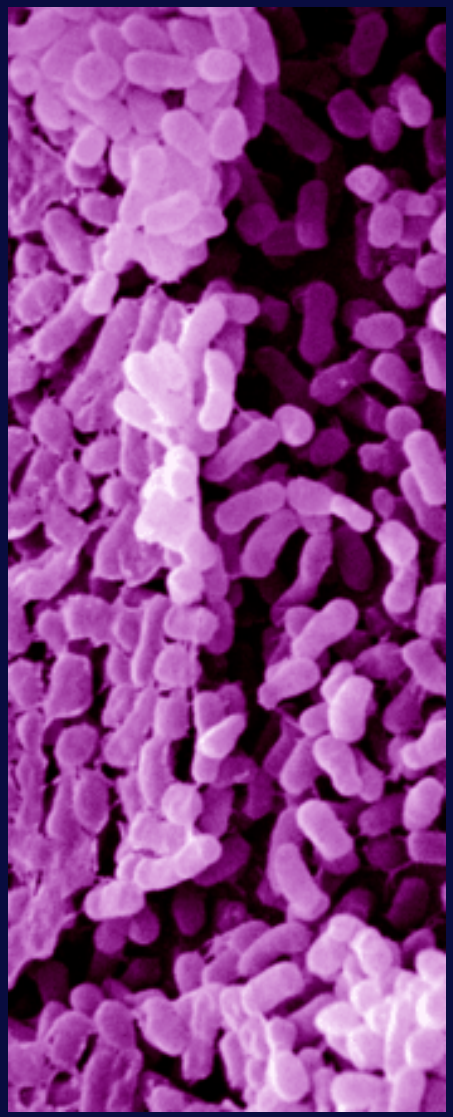
RESISTANCE TO ANTIBIOTICS:

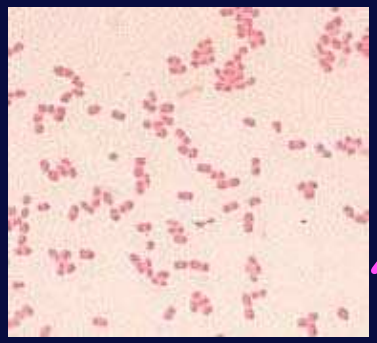
- -MULTIDRUGRESISTANT ISOLATES
- -ADQUISITION OF GENES

INCREASE MORBI-MORTALITY & COSTS

Acinetobacter baumannii:
EMERGING PATHOGEN IN NOSOCOMIAL INFECTIONS

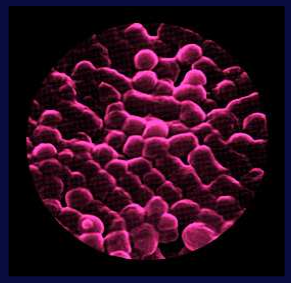






Acinetobacter: Description

COCCOBACILLI
GRAM-NEGATIVE
NON-MOTILE
CATALASE-POSITIVE
OXIDASE-NEGATIVE
NON-FERMENTER
STRICTLY AEROBIC
GC CONTENT 38-47%



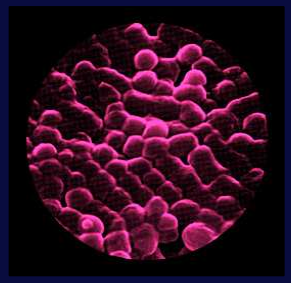
Acinetobacter Ecology

1.Natural environment (water, soil): able to survive on dry environmental surfaces for a prolonged time

2.Food: dairy products, meat, fish...

3.Healthy humans: often colonized in the pharynx, on the skin and in the rectum

4. Important cause of nosocomial infection inpatients attended at hospitals: spread faciliated by its ability to contaminate floors, sinks, tabletops,doors, patients charts, telephone handless, mattresses, pillows, bed linens and curtains.



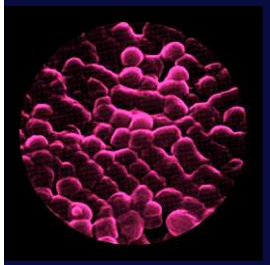
Acinetobacter Ecology

DIFFERENT SPECIES ⟶ DIFFERENT ENVIRONMENT

1. HOSPITALS: *A. baumannii*, sp.3, sp.13TU
   (most of the isolates show resistance to antibiotics)

2. SKIN AND FOODS: *A. johnsonii, A. lwoffii, A. radioresistens* (susceptible to antibiotics)

3. SOIL, WATER AND NATURAL ENVIRONMENT:
   A. calcoaceticus, A. johnsonii (susceptible to antibiotics)

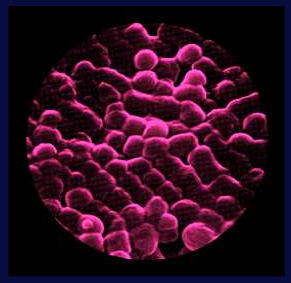


Acinetobacter clinical isolates

EPIDEMIC & ENDEMIC SPECIES:

A. baumannii
sp.3
sp.13TU
→ *A. baumannii* complex

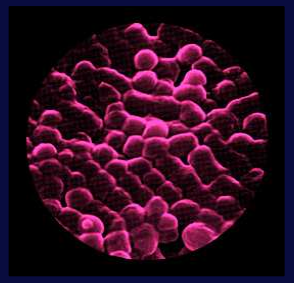
RARE INFECTIONS CAUSED BY: *A. calcoaceticus, A. haemolyticus, A. junii, A. johnsonii, A. lwoffii, A. radioresistens, A. ursingii*



Acinetobacter virulence factors

1. Polysaccharide (capsule)
2. Adhesion
3. Production of enzymes that damage soft tissues
4. Lipopolysaccharide of the cell envelope and lipid A
5. Endotoxins ?

6. *Slime* production by virulent strains:
    a) Inhibit neutrophils migration
    b) Increases virulence in mixed infections



Acinetobacter at hospitals

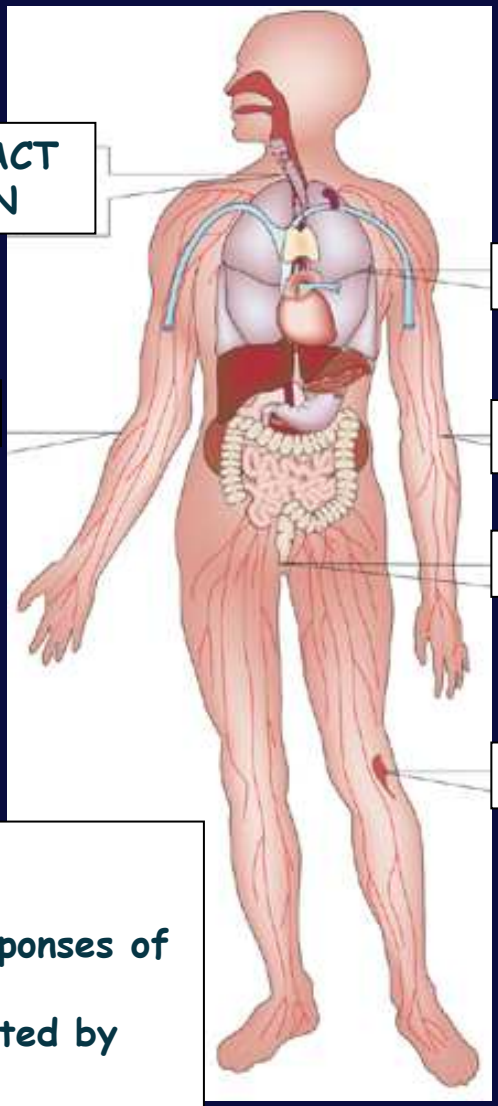
1. PERSISTENCE

2. ANTIBIOTIC RESISTANCE

3. NOSOCOMIAL OUTBREAKS

RESPIRATORY TRACT COLONIZATION

SKIN COLONIZATION



PNEUMONIA

BACTERIEMIA

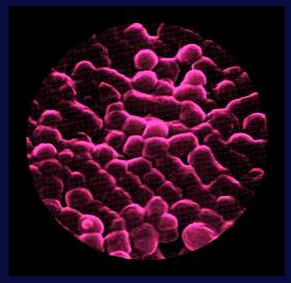
URINARY TRACT INFECTION

WOUND INFECTIONS

COLONIZATION:
- Adherence to host cells
- Resistance to immune responses of skin and mucosal surfaces
- Biofilm production (regulated by "*Quorum sensing*")

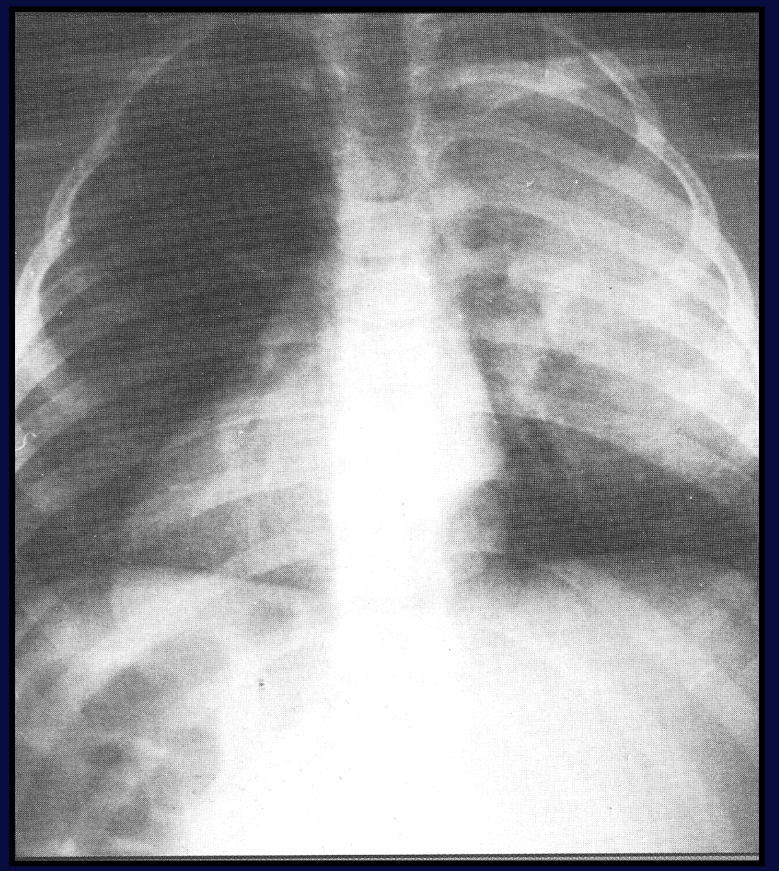
INFECTION:
- Inflamatory response
- Citotoxicity
- Iron uptake
- Resistance to complement activity

SURVIVAL IN THE NATURAL ENVIRONMENT:
- Resistance to dry conditions, disinfectants and antibiotics
- Use of different metabolic sources
- Biofilm production

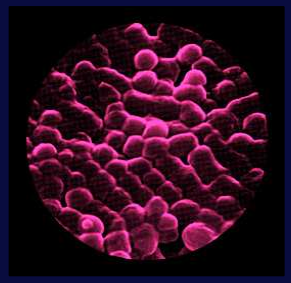


Acinetobacter infections

1. Pneumonia, Chronic bronchitis
2. Bacteremia
3. Meningitis
4. Urinary tract infections
5. Endocarditis….



Countries in red: high rate of Acinetobacter infections



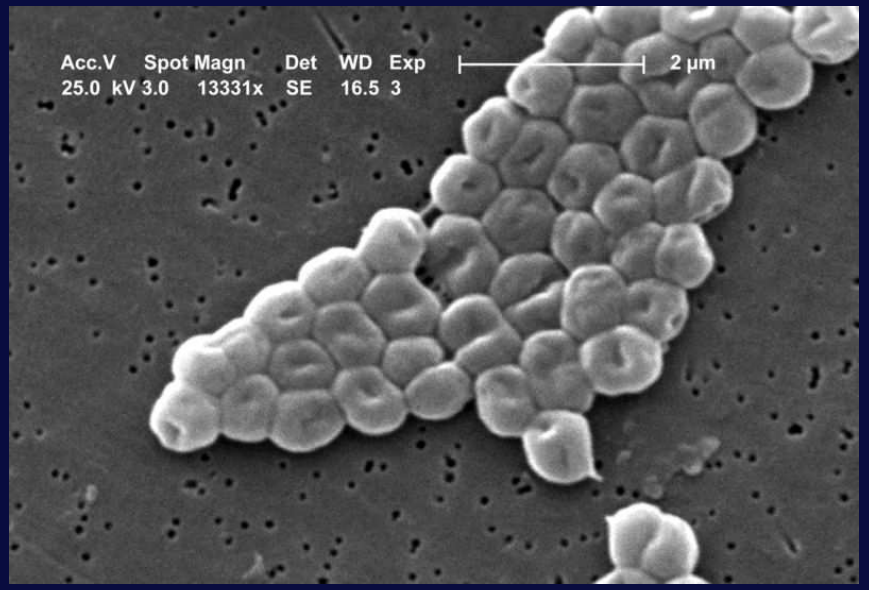
Acinetobacter Risk factors

1. ELDERLY PEOPLE
2. IMMUNOCOMPROMISED PATIENTS
3. UNDER BROAD-SPECTRUM ANTIBIOTIC TREATMENT
4. WITH MECHANICAL VENTILATION..
5. HOSPITALIZED FOR EXTENDED PERIODS
6. BURN WOUNDS

Acinetobacter

¿is it the new gram-negative SAMR?

- INFECTIONS IN IMMUNOCOMPROMISED PATIENTS
- MULTIRESISTANCE
- CAUSE EPIDEMIC OUTBREAKS
- PERSISTANCE



INCREASE OF THE COST OF HOSPITAL CARE

TABLE 1. Survey of global susceptibility of *A. baumannii* to selected antibiotics

Geographic area	Location/study[a]	Yr	Susceptibility (%) to[b]:										Reference
			FEP	CAZ	CIP	GEN	IMP	LVX	SAM	MEM	TZP	SXT	
North America	SENTRY	2001–2004	57	54	54		89		71	84			50
	United States (hospital isolates)/ SENTRY	1998–2003	63	62	61	64	93				63		172
	United States (hospital isolates)/ MYSTIC	2003	63	64	59	63	92	60		87	61		162
	United States (non-ICUs)	2001	47	45	35	44	93	45		85	58		87
	United States (ICUs)	2001	56	49	45	53	96	54		91			87
	United States (ICUs)/SENTRY	2001	51	57	53	53	81			79	59		191
	United States (ICUs)/TSN	2000–2002	44	42	40	47	87	44		66	54	51	83
	Canada (ICUs)/TSN	2000–2002	67	71	72	73	96	61		94	71	75	83
	United States/ICUSS	2000	66	55	43		95		78		79		48
Europe	SENTRY	2001–2004	44	40	39		74		48	70			50
	Italy (ICUs)/TSN	2000–2002	18	26	21	23	78	14		75	35	44	83
	France (ICUs)/TSN	2000–2002	28	35	38	49	94			68	75	45	83
	Germany (ICUs)/TSN	2000–2002	74	67	75	82	96	82		96	82	84	83
	Sweden (ICUs)	1999–2000			89		96				40	96	62
	Spain (hospital isolates)	2001	49	24	7	15	60	10	58	49	17	32	147
	United Kingdom and Ireland (bacteremia)	2001–2002		35	79	83	100				87		161
	Italy (respiratory isolates)	1997–1999	55	42	48	54	87			84	49	57	40
Asia/Pacific	SENTRY	2001–2004	58	58	55		74		59	73			50
	Korea (hospital isolates)	2003	59	45	42	36	87		53	75	58	43	100
	China (ICUs)	2002	70	65	66		92		80		70		220
	Japan (hospital isolates)	2002	85	89			95		97				76
	Taiwan (hospital isolates)/TSAR	2000	40	27	31	18	98				26	22	97
Latin America	SENTRY	2001–2004	36	32	35		86		52	84			50
	Brazil/SENTRY	2001	37	29	33	39	98	33		97	31	37	83
	Argentina (hospital isolates)	2001–2002	37	23			85	17	32		22		19

[a] SENTRY, SENTRY Antimicrobial Surveillance Program; MYSTIC, Meropenem Yearly Susceptibility Test Information Collection; TSAR, Taiwan Surveillance of Antimicrobial Resistance; ICUSS, Intensive Care Unit Surveillance System; TSN, The Surveillance Network; TSAR, The Taiwan Surveillance of Antimicrobial Resistance Program.

[b] FEP, cefepime; CAZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; IMP, imipenem; LVX, levofloxacin; SAM, ampicillin-sulbactam; MEM, meropenem; TZP, piperacillin-tazobactam; SXT, trimetropim-sulfamethoxazole.

RESISTANCE TO CARBAPENEMS (last therapeutic option in infections caused by resistant isolates)

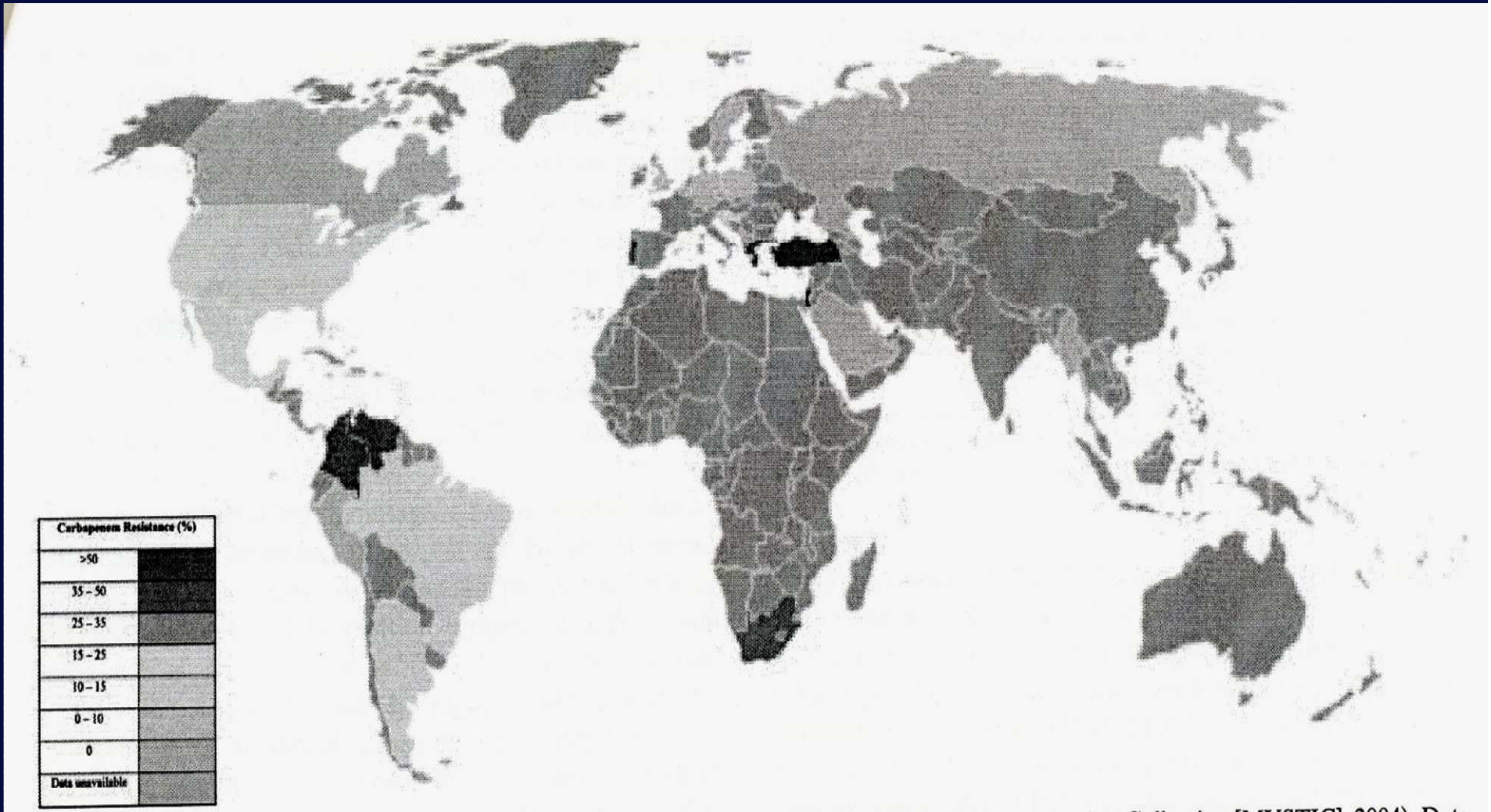
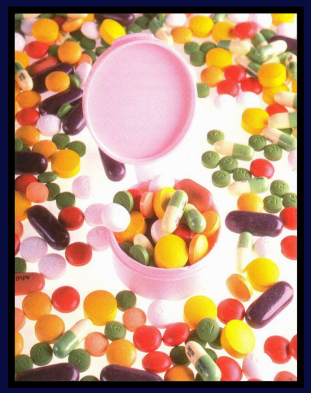


FIG. 1. *Acinetobacter* isolates resistant to carbapenems (Meropenem Yearly Susceptibility Test Information Collection [MYSTIC], 2004). Data were extracted from the MYSTIC database (www.mystic-data.org).



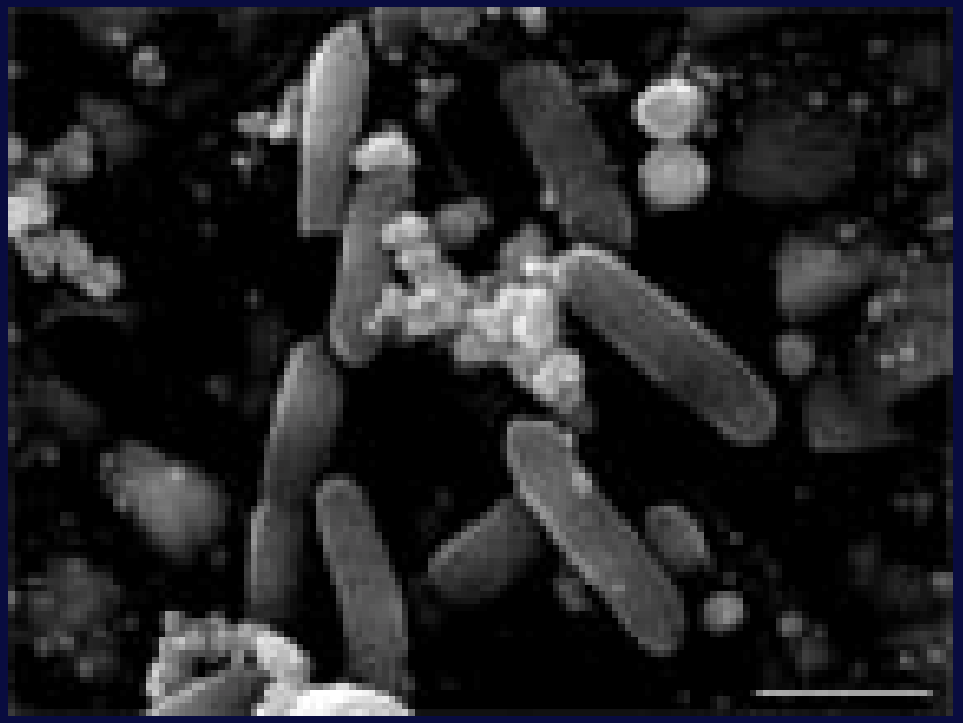
TREATMENT OF INFECTIONS
Pseudomonas aeruginosa & Acinetobacter baumannii

- **AMINOGLICOSYDES**: AMIKACIN, TOBRAMICIN AND GENTAMICIN

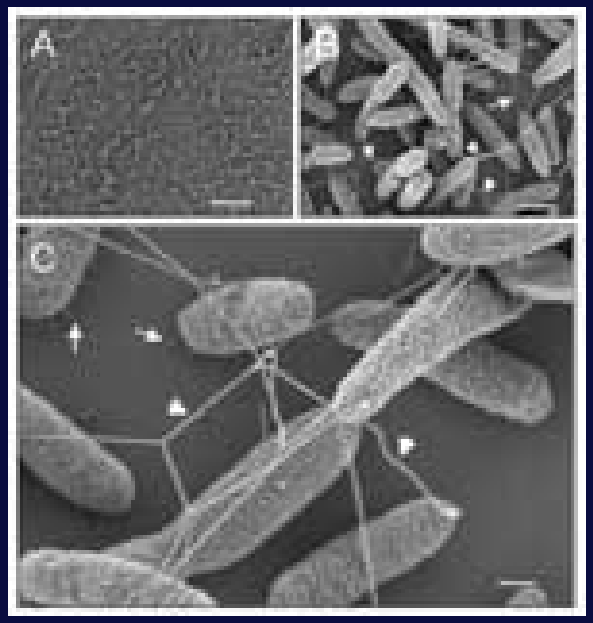
- **BETALACTAMS:**
  - CEFTAZIDIM, PIPERACILLIN, TICARCILLIN
  - CEFEPIME, AZTREONAM
  - IMIPENEM, MEROPENEM
  - AMPI/SULBACTAM, AMOXI/CLAVULANIC ACID
    PIPER/TAZOBACTAM

- **FLUOROQUINOLONES**: CIPROFLOXACIN, NORFLOXACIN, LEVOFLOXACIN

Stenotrophomonas



-MOST IMPORTANT SPECIE :*Stenotrophomonas maltophilia* (named *Pseudomonas maltophilia, Xanthomonas maltophilia*)



-OTHERS: *S. africana,S. nitritireducens, S. acidaminiphilia & S. rhizophila*

Stenotrophomonas maltophilia

-MOTILE

-DIFFERS FROM OTHER NON-FERMENTERS : LISINE +
DNAse +
OXIDASE -

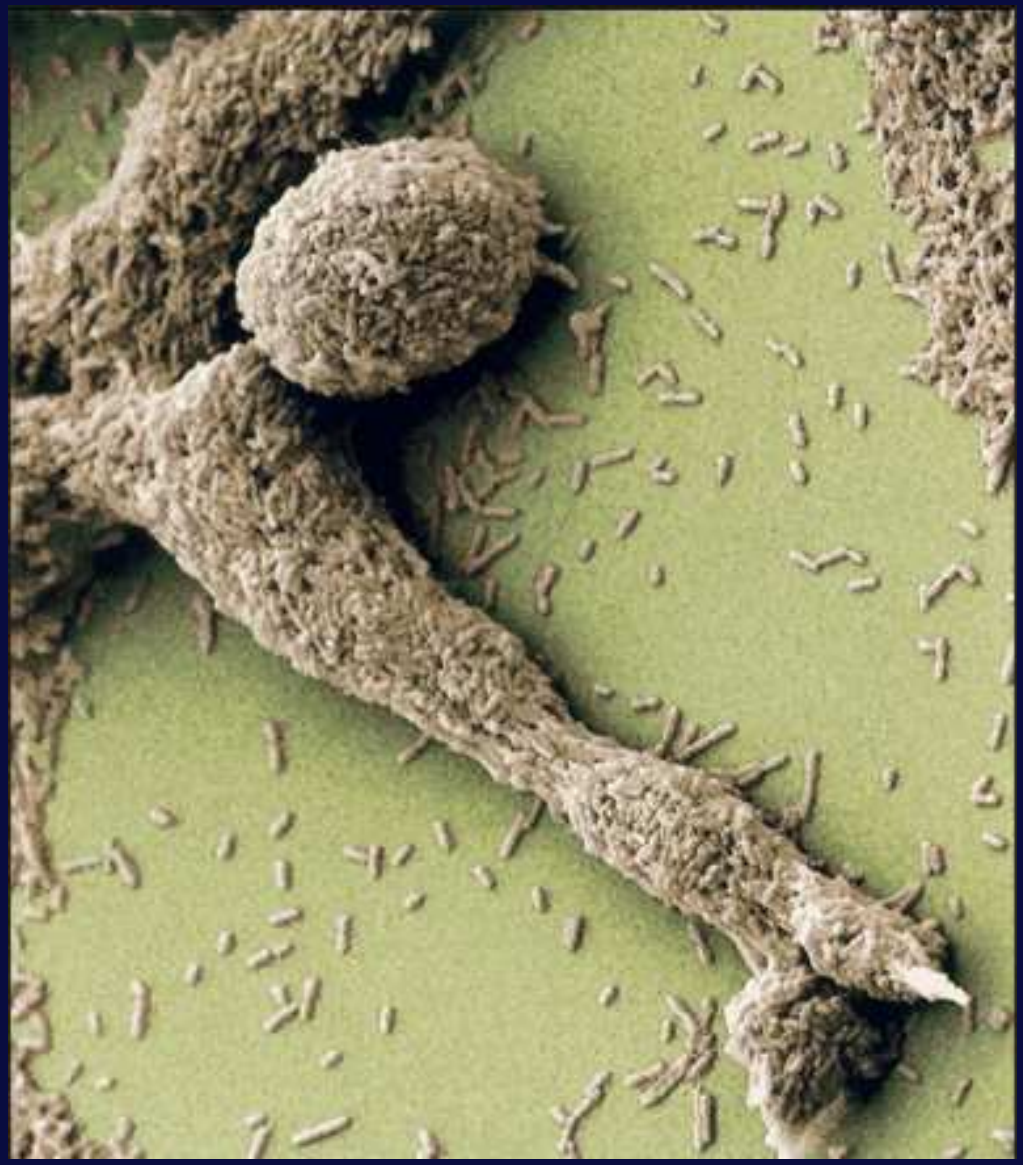
-YELLOW OR LAVENDER COLONIES ON BLOOD AGAR

-UBIQUITOUS

-EMERGING PATHOGEN

-FREQUENTLY ISOLATED FROM
RESPIRATORY TRACT SPECIMENS

VIRULENCE FACTORS: *biofilms*



NOSOCOMIAL INFECTIONS

-COLONIZATION IN PATIENS AFFECTED BY CYSTIC FIBROSIS
-PNEUMONIA
-BACTEREMIA
-ENDOCARDITIS
-UNINARY TRACT INFECTIONS
-MENINGITIS
-WOUND INFECTIONS

MORTALITY RATE 50%

RESISTANCE

-INTRINSIC TO:

- AMINOGLUCOSYDES & MOST BETALACTAMS (β-LACTAMASES L1 & L2)
- SUSCEPTIBLE TO TRIMETOPRIM-SULPHAMETOXAZOL

-SUSCEPTIBLE TO COLISTINE & POLIMIXIN B

- AUTOMATED EQUIPMENTS DO NOT SHOW ACCURACY WHEN TESTING *S. maltophilia* RESISTANCE PROFILES