Pseudomonas & Acinetobacters (and other non-fermenters)

Are they pathogens or are they not?

- a complex **mixture of opportunistic pathogens** of plants, animals, and humans
- are **ubiquitous organisms** found in soil, decaying organic matter, vegetation, and water. Unfortunately, they are also found throughout the hospital environment.

The group characterization

- small, **gram-negative rods** typically arranged in pairs, **simple nutritional needs**
- **obligate aerobe**; **glucose oxidizer (enterobacteria – glucose fermenters)**; simple nutritional needs, ussually **oxidase positive** (enterobacteria – negative)

Virulence (factors)

- **they are not very well characterized**
- **virulence factors – structural components** (e.g. capsule – suppresses neutrophil and lymphocyte activity, pili – adhesin, LPS – endotoxin activity,), **toxin & enzymes** (e.g. exotoxins – tissue destruction, hemolysins), **ATB and desinfectant resistance**

Colonization and/or infection

- transiently **colonize** the respiratory and gastrointestinal tracts of **hospitalized patients**, particularly **those treated with broad-spectrum antibiotics**
- most of the infections in humans are caused *Pseudomonas aeruginosa, Burkholderia cepacia, Stenotrophomonas maltophilia, Acinetobacter baumannii*

Treatment, prevention & control

- combined use of effective antibiotics

(e.g., **aminoglycoside** and β-lactam antibiotics); **monotherapy is generally ineffective**

preventing contamination of sterile medical equipment and nosocomial transmission; unnecessary use of broad-spectrum antibiotics can select for resistant organisms

Species and infection

- ***Pseudomonas aeruginosa***
- pulmonary infections
- bacteremia
- primary skin infections
- urinary tract infections
- ear and eye infections

Virulence factors of P. aeruginosa

- many virulence factors

Structural components

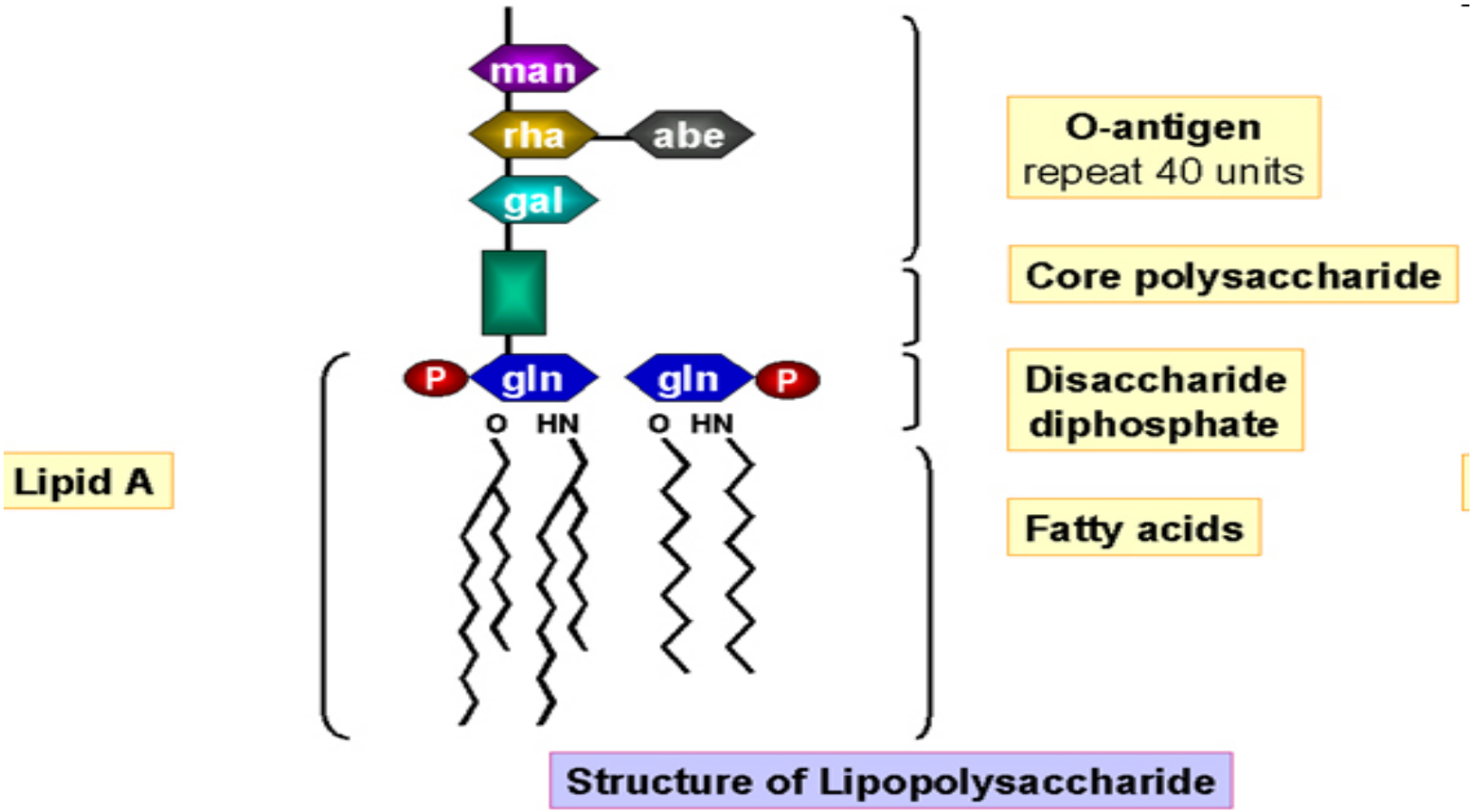
* adhesins – pili, nonpilus (binding to epith.cells)

* polysacharide capsule – alginate (anchor bacteria to epit.cells, protect from phagocytosis and complement and antibiotics , biofilm in vivo)

* endotoxin (next slides)

* pyocyanin – blue pigment, toxin forms of oxygen (superoxide, hydrogen peroxide)– tissue damage

Endotoxin is a lipopolysaccharide integrated in the outer membrane of Gram-negative bacteria



-Lipid A embedded in outer membrane, core and O antigen portions extending from the bacterial surface

http://pathmicro.med.sc.edu/fox/lps.jpg

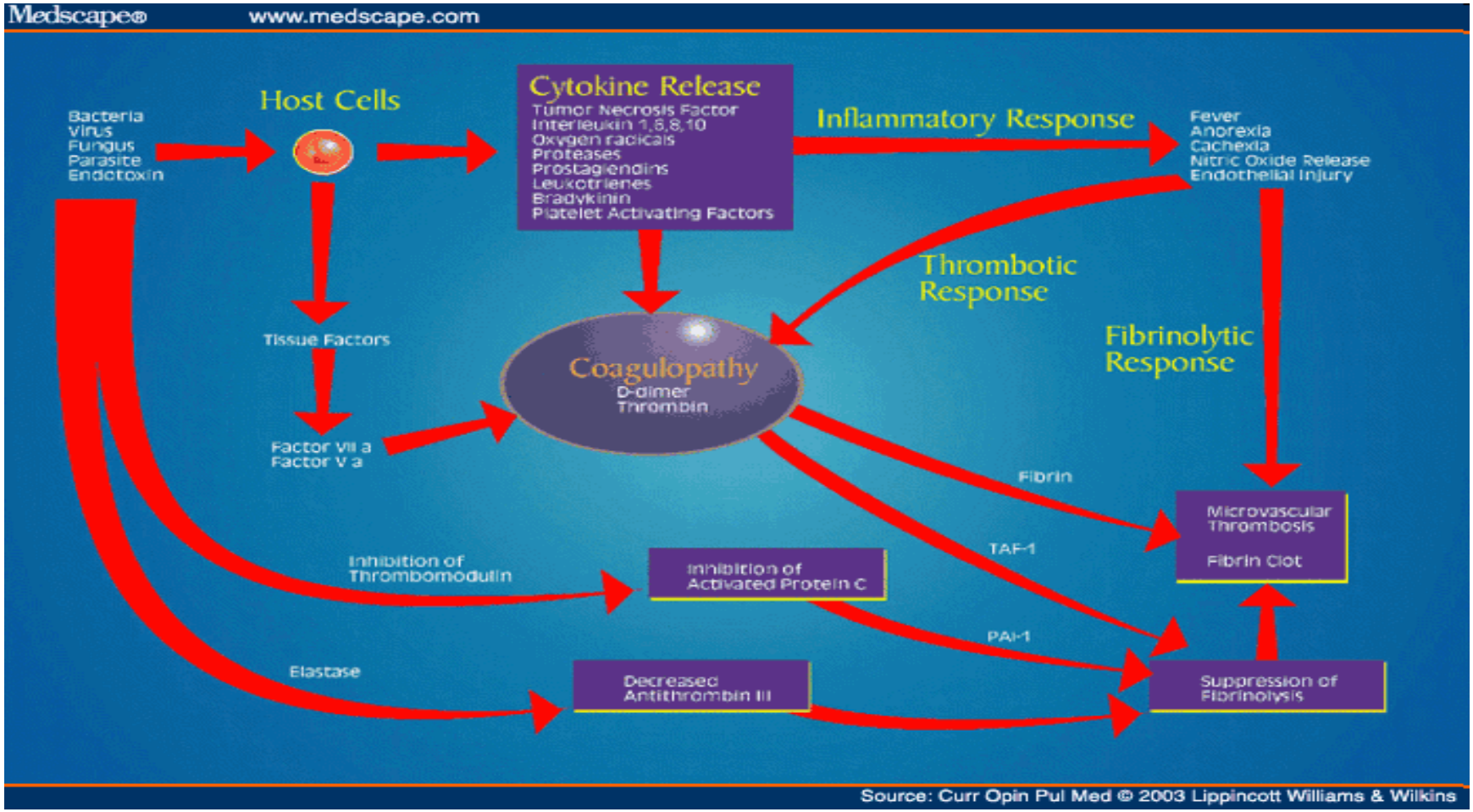
Endotoxin properties

- Lipid A is toxic portion of endotoxin (activate complement and cytokines cascade)
- Exerts its effect only when bacteria lyse (result of complement atack, ingeston and killing by phagocytes or certain antibiotics)
- Lipid A become toxic if its in too high concentration
- Consequences – local inflamation (activation of complement) and systemic response (septic shock)

Endotoxin and septic shock

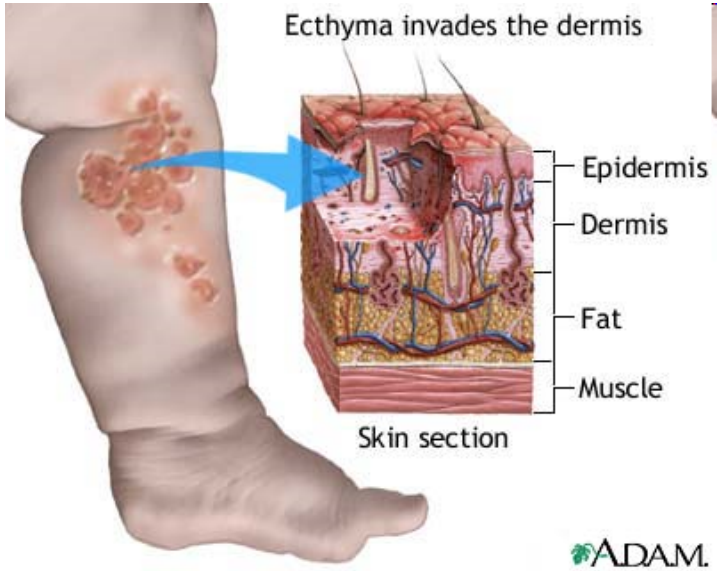
- shock - clinical term, a set of events that lead to collapse of the circulatory system and can result in multiorgan system failure
- etiology - variety of insults but bacterial infection is the most common cause
- 1. step - production of cytokines by monocytes, macrophages and other cells
- 2. step - activation of complement cascade
- 3. step – activation of coagulation cascade

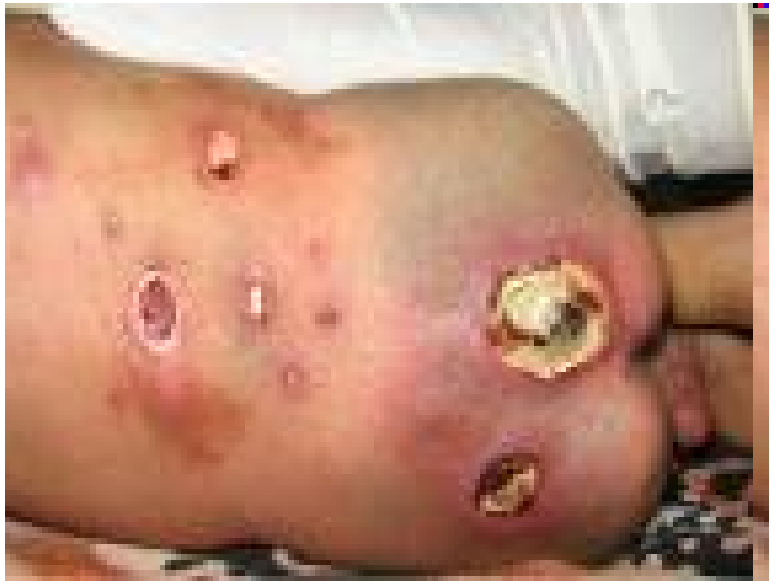
Diagram of septic shock



Toxin and enzymes

- exotoxin A, S (blocking elongation factor inhibit proteosynthesis)
- Elastase (damage elastin in tissue - lung parenchymal damage, hemorragic lesions proteases – figures below, phospholipase C – tissue damase mediate and disrupting of inflamatory response





ecthyma granulosum

Treatment

- resistant to most antibiotics
- monotherapy is not usually successful
- drug of first choice:

Aminoglycoside – AMG (gentamicin, tobramycin, amikacin, netilmicin – depending on local patterns of susceptibility)

and

Antipseudomonal penicillin (ticarcilin, piperacilin)

- Alternative drugs:

AMG+ CEF III (CTZ), + karbapenems (IMI,MER),+monobactams (AZT)

fluorochinolones (CIP) + piperacillin, + CEF III (CTZ), + CEF IV cefepime

Resistant determinants

- most significant – efflux mechanism – ATB are actively pumped from the bacterial cell (range of ATB – TET,CHLORAMPH,QUINOLONES, β LACTAMS
- β lactamases – mostly on chromosome (less on plasmids and transposons than in enterobacteria)
- resistance to carbapenemes – not linked to resistence to β lactamas but connected with lost of a porin (D2) – specific channel
- AMG – non-enzymatic (reduced OMP permeability, efllux pump – active transport need energy – ATP or proton motif force)
- Quinolones – mutation in DNA gyrase, also efflux

Main types of bacterial drug efflux pumps

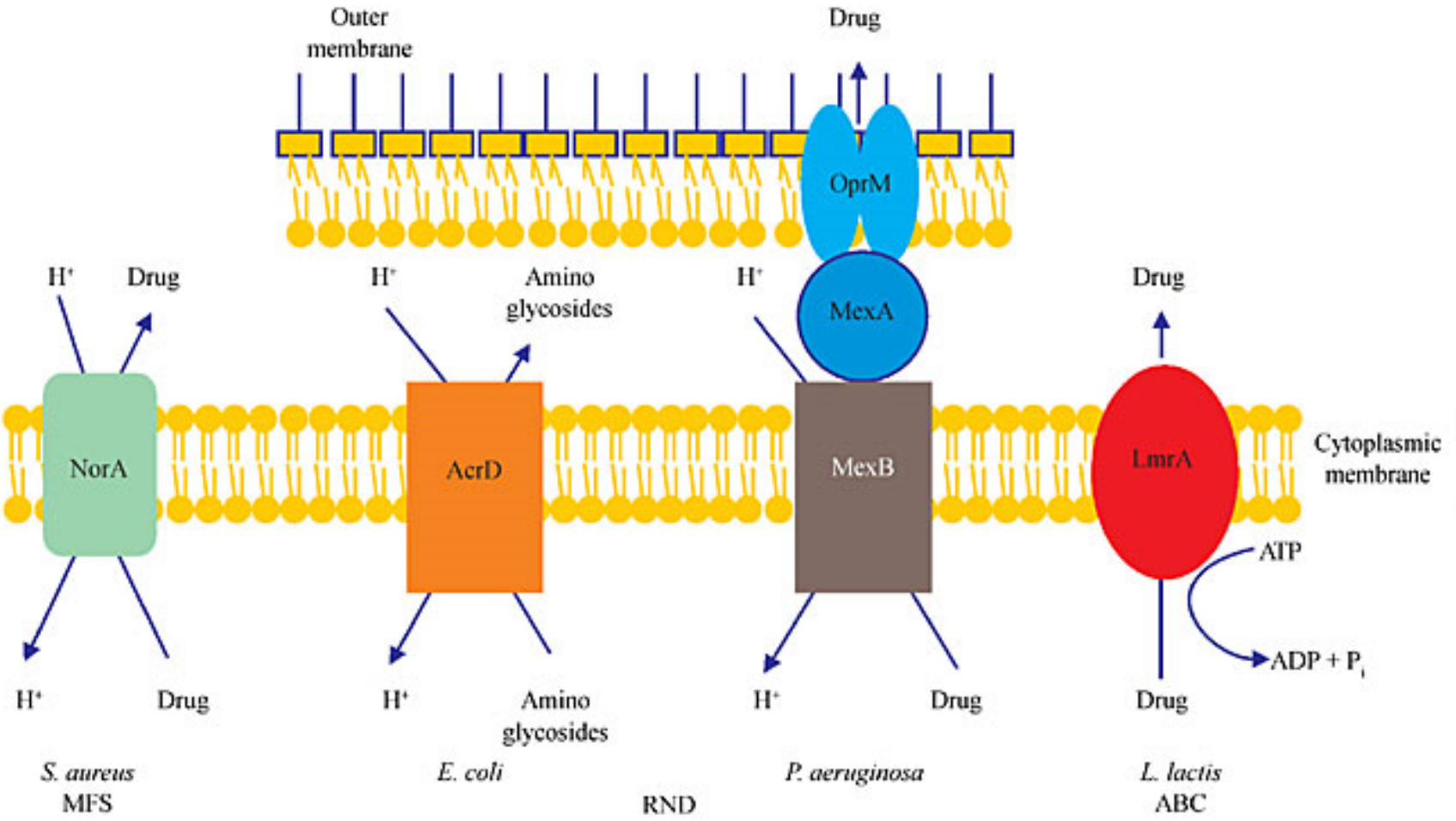


Figure 1. Schematic illustration of the main types of bacterial drug efflux pumps shown in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Lactobacillus lactis*. Illustrated are NorA, a member of the major facilitator superfamily (MFS); AcrD and MexAB-OprM, two members of the resistance-nodulation-division (RND) family and LmrA, a member of the ATP-binding cassette (ABC) family. All systems extrude drugs in an energy-dependent manner, either by using proton motif force or ATP. The two other types of efflux systems found in bacteria, multidrug and toxic compound extrusion (MATE), and small multidrug resistance (SMR), look structurally similar to the MFS but are designated as distinct families, based on phylogenetic diversity (MATE) or size (SMR).

Genes encoding protein components of efflux pumps

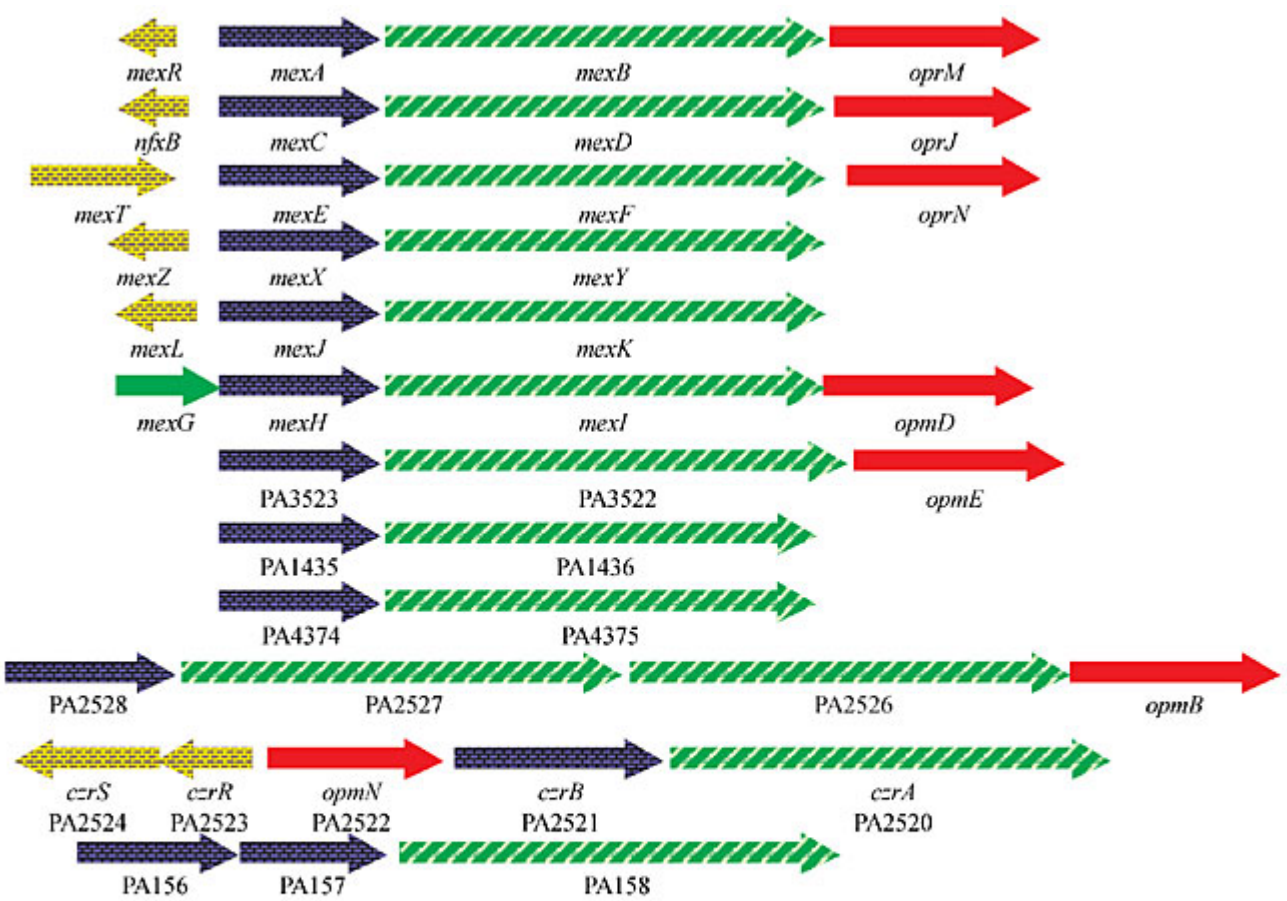


Figure 2. Proposed resistance-nodulation-division (RND) efflux pumps encoded by the *Pseudomonas aeruginosa* genome. Genes encoding protein components of characterized efflux pumps are denoted by gene names and genes encoding protein components of uncharacterized pumps are labeled with the PA numbers assigned in the annotated *P. aeruginosa* genome sequence. RND transporter-encoding genes are depicted as cross-hatched yellow/green arrows, major facilitator superfamily-encoding genes as blue arrows and regulatory genes as yellow arrows. An additional membrane protein-encoding gene required for function of the MexGHI-OpmD efflux pump is shown as a green arrow. Outer membrane channel protein designations are those proposed by Dr. R.E.W. Hancock from the University of British Columbia (http://cmdr.ubc.ca).

Species and infection

- ***Acinetobacter baumannii*** – serious nosocomial pathogen of opportunistic infections (e.g. pulmonary)

Resistance to carbapenems in nosocomial isolates of *Acinetobacter baumannii* in ČR

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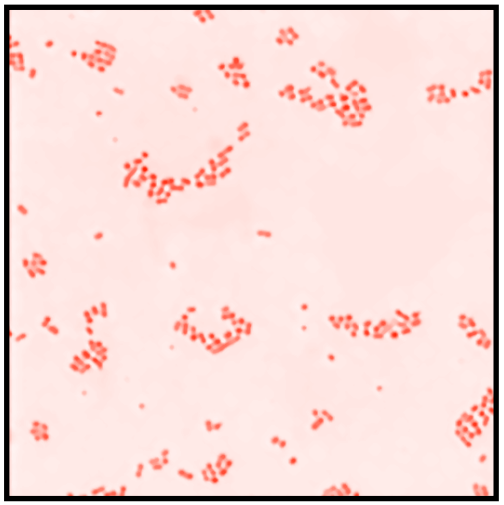
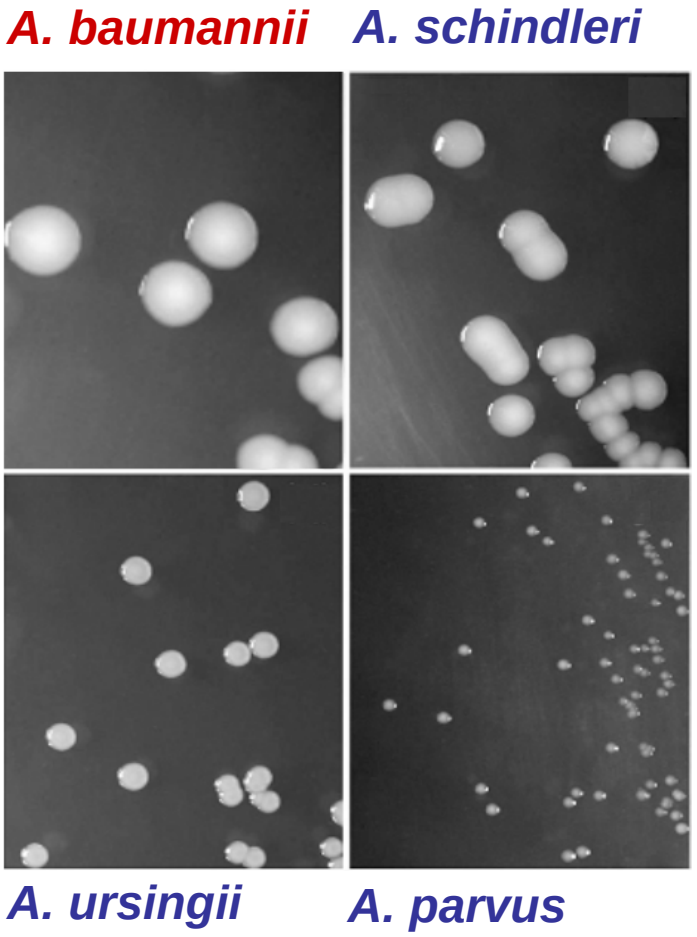
Rod *Acinetobacter*

- **Gramnegative, aerobic, non-motile**
- **Ubiqitous in nature**
- **Low virulence**
- **Agent of nosocomial infections of critically ill patients**
- **Outbreaks in ICUs**



Clinically significant Ac species

A. baumannii
A. ursingii
A. schindleri
A. parvus
A. haemolyticus
A. junii
A. johnsonii
A. lwoffii
A. radioresistens



Acinetobacter baumannii

- **Clinically end epidemiologically most significant**
- **Usually contain all nosocomial isolates**
- **All epidemiological and multidrug resistant strains are *A. baumannii***

Resistance *of A. baumannii* to antibiotics

Usually resistant to

- **β-laktamovým antibiotikům** (piperacilin, cefalosporiny III.generace, kombinace s inhibitory β-laktamáz)
- **aminoglykozidům** (gentamicin, amikacin)
- **fluorochinolonům** (ciprofloxacin)
- **tetracyklinům, ko-trimoxazolu**

Karbapenemy (imipenem, meropenem) first drugs of choise for MDR isolates

In late 90 all over the world increasing resistance to the group of ATB

Goal of the project

- Isolations of representative isolates from ICUs (ARO, JIP)
- Determination of relation between resistance to carbapenems and clonality of the isolates

Methods

- Isolates collection
- Biochemical identification
- Susceptibility test – disk diffusion method
- Macrorestriction analysis of DNA
- Classification of the isolates

Collection of isolates

- **Only clinical samples (sputum, blood, urine …)**
- **only ARO and JIP**
- **Single-patient isolate**
- **At most 10 isolates from a Dept.**

Totally: **194 isolates**

15 towns

20 hospitals

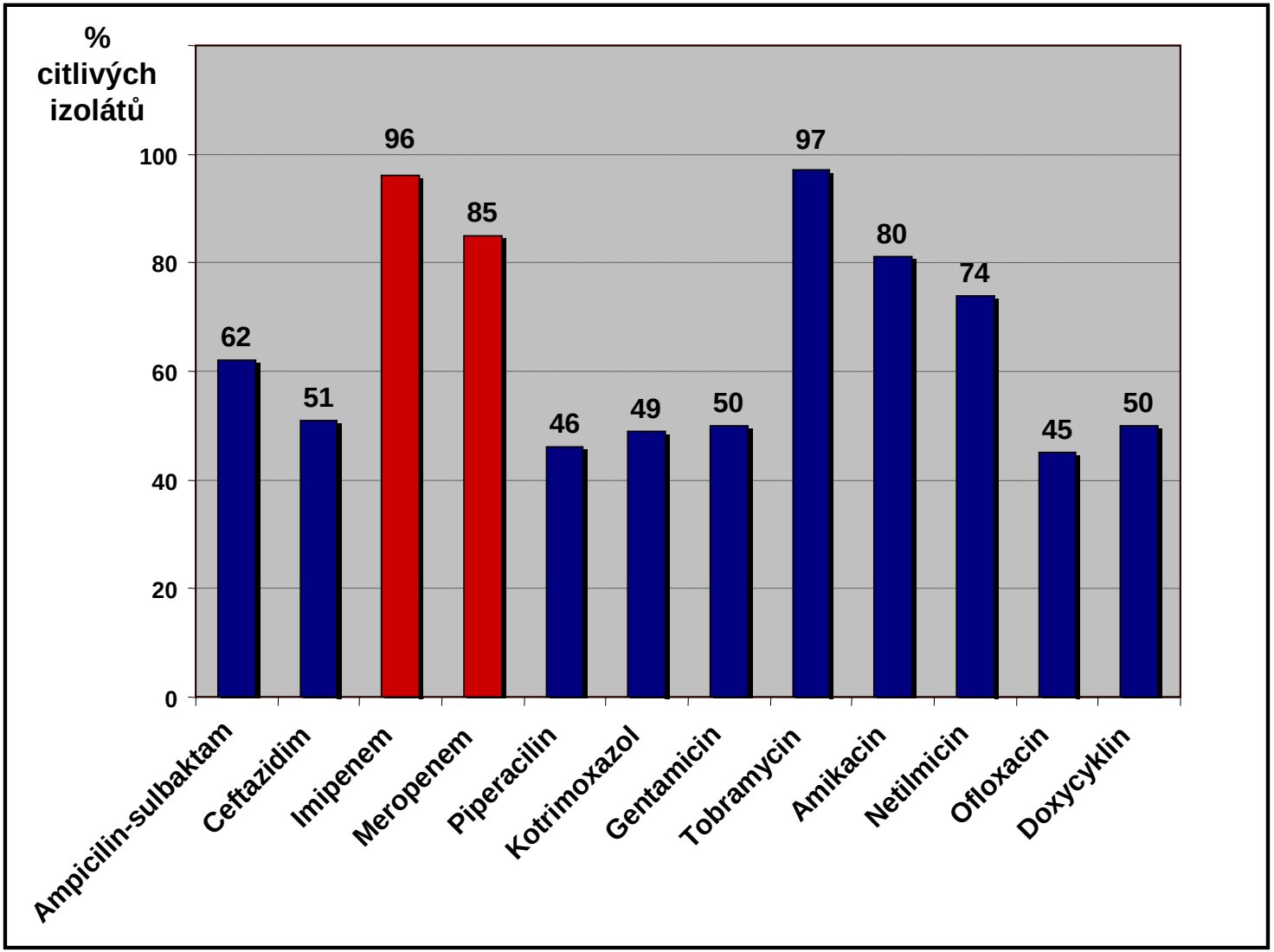
60 departments JIP/ARO

The isolates origin

Antibiotic analysed

β-laktamy	• Piperacilin • Ceftazidim • Ampicilin-sulbaktam • Imipenem • Meropenem
Aminoglykozidy	• Gentamicin • Tobramycin • Amikacin • Netilmicin
Fluorchinolony	• Ofloxacin
Tetracykliny	• Doxycyklin
Sulfonamidy	• Kotrimoxazol

Susceptibility (194 isolates)



Typing
(194 isolates)

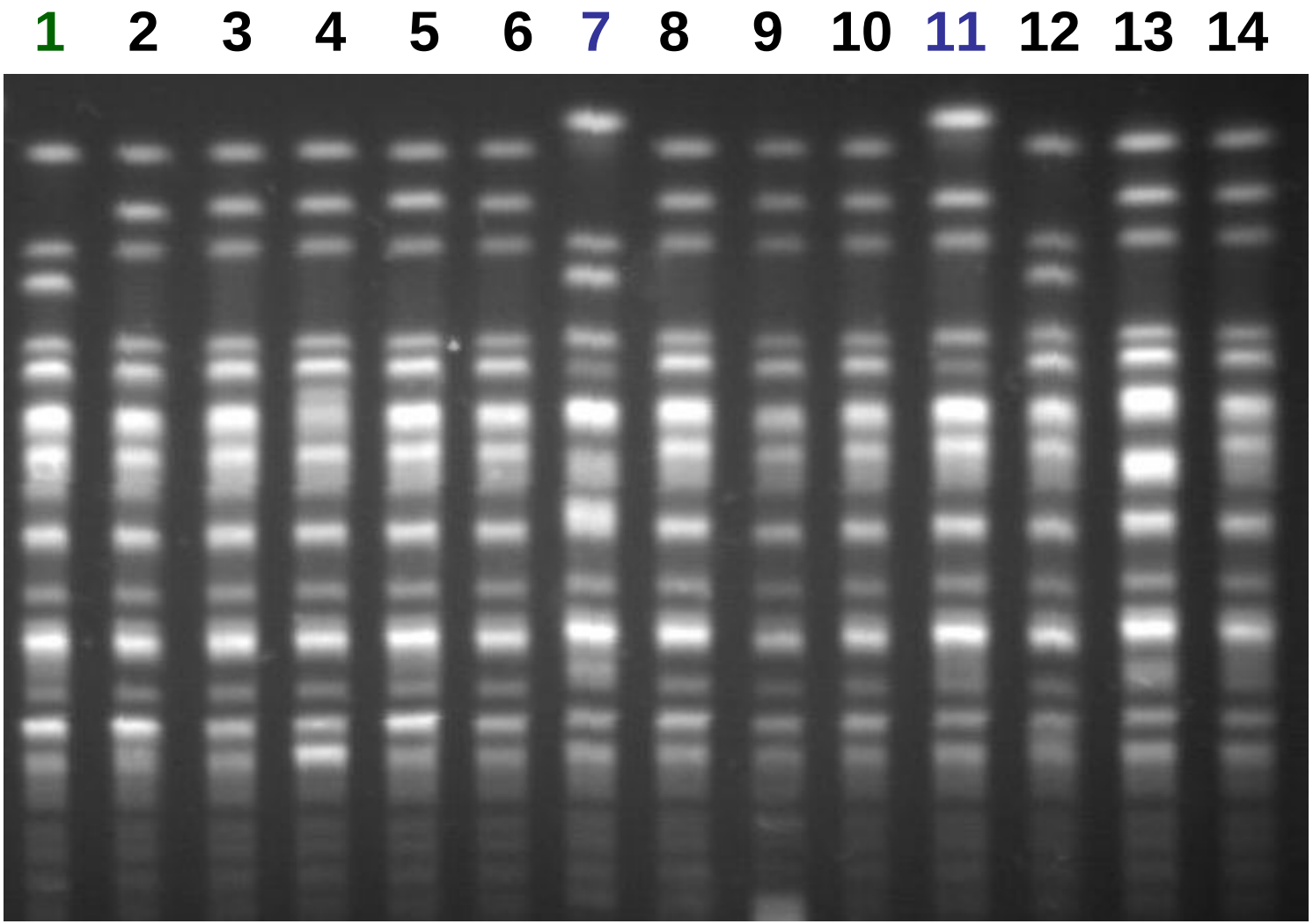
Typing methods

- **Susceptibility**
- **Biotyping**
- **Makrorestriction analysis DNA**

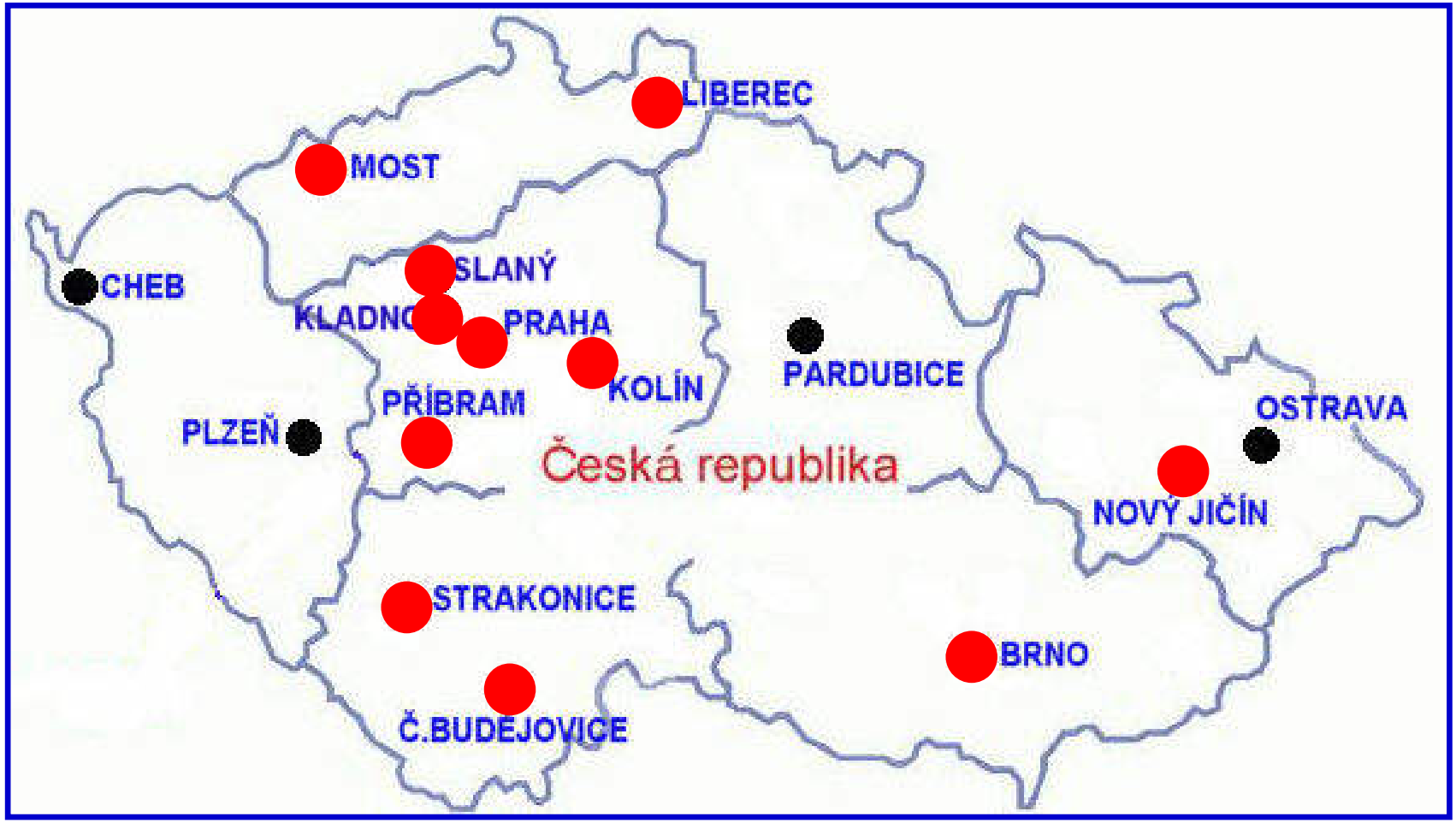
Classification of the isolates

▪ **MR epidemic clone *A. baumannii***	**93**
other MR kmeny	**9**
▪ **Susceptible isolates**	**92**

Makrorestriction analysis of epidemic clone of *A. baumannii*

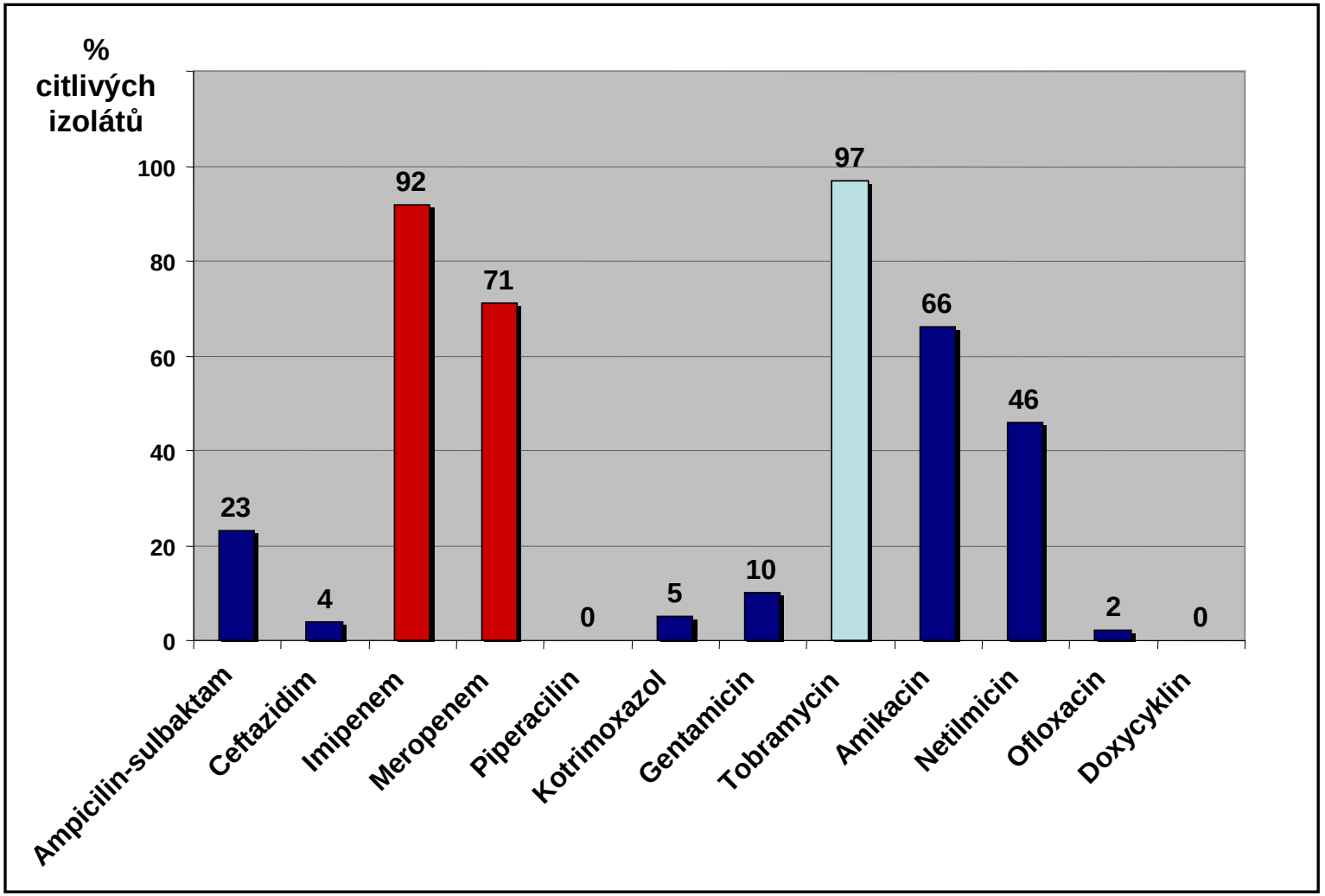


Původ izolátů epidemického klonu



● Prokázaný výskyt MR epidemického klonu *A. baumannii*

Susceptibility to ATB (epidemic clone)



Species and infection

- ***Burkholderia cepacia***
  Pulmonary infections: Range from colonization to bronchopneumonia primary in patients with cystic fibrosis or chronic granulomatous disease
  Opportunistic infections: Urinary tract infections in catheterized patients; bacteria in immunocompromised patients with contaminated intravascular catheters

Species and infection

- ***Stenotrophomonas maltophilia***
  Opportunistic infections: A variety of infections (most commonly pulmonary and urinary tract) in immunocompromised patients previously exposed to broad-spectrum antimicrobial therapy

References

- Murray et al. Medical Microbiology, 2007
- Jawetz, Melnick and Adelbergs Medical Microbiology, 2007
- web references
- Maixnerová M., Melter O., Nemec A. Acinetobacter baumannii in ČR