Pseudomonas aeruginosa: from environment to humans

Finovi

Thursday 9th december 2010, Lyon

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University of Lyon 1

Pseudomonas aeruginosa

- Carle Gessard (1850-1925)

PHYSIOLOGIE PATHOLOGIQUE. — *Sur les colorations bleue et verte des linges à pansements*. Note de M. C. Gessard, présentée par M. Pasteur.

« Deux cas de coloration bleue et verte des pansements se produisaient en octobre dernier, dans le service de M. le Dr Chauvel, au Val-de-Grâce. Les linges me furent remis, et j'entrepris de vérifier l'origine parasitaire du phénomène, par la méthode des cultures de M. Pasteur. J'ai pu isoler de la sorte un organisme qui, après un grand nombre d'ensemencements successifs, se montre constant dans sa forme et dans sa fonction physiologique, la production de pigment, pour les différents liquides de culture. Cet organisme est incolore, globuleux, de 1 à 1,5 millième de millimètre; il est aérobie et très mobile. On le cultive bien, entre 35° et 38°, dans l'urine neutralisée, la décoction de carottes. Il se développe également dans la salive, la sueur, les liquides albumineux, sérosité de vésicatoire, d'hydrocèle. La matière colorante bleue sécrétée est la pyocyanine de M. Fordos (2),

(1) Ce travail a été fait au laboratoire de M. Schützenberger, au Collège de France.
(2) *Comptes rendus*, t. LI, p. 215, et t. LVI, p. 1128.

« On the Blue and Green Coloration of Bandages »

- C.R.Séances Acad. Sci., 1882; p. 536-538 (Série D)

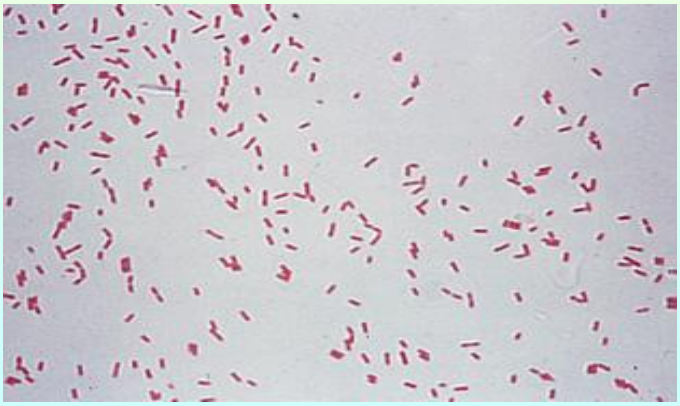
Pseudomonas aeruginosa

- Pseudomonas: *false unit*, from the Greek pseudo and the Latin monas
- aeruginosa: *copper rust (*Latin)

pyocyanin

Pseudomonas aeruginosa

- Gram negative bacilli
- Unipolar motility

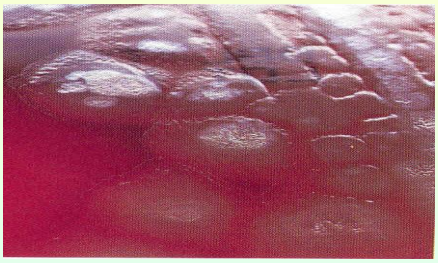


Pseudomonas aeruginosa

- Strict aerobes

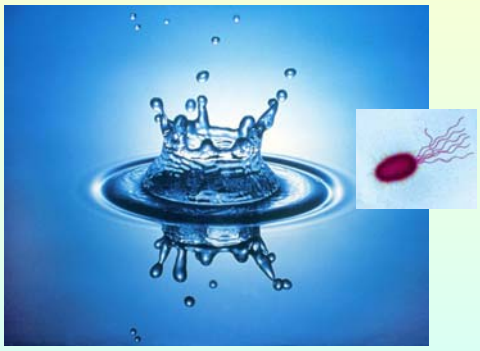
Pseudomonas aeruginosa

- **Identification**



Pseudomonas aeruginosa

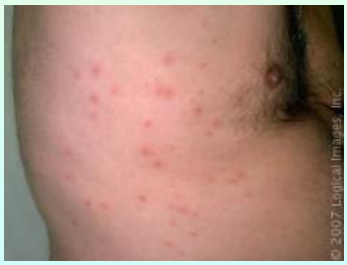
Freshwater: from lakes to hot tubes



inhalation,
aspiration,
direct application to intact
or injured skin,
invasion of respiratory tract

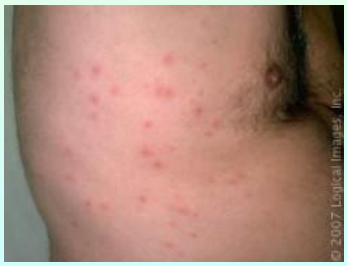
Skin and soft tissues infections

- ***Pseudomonas*** **dermatitis/folliculitis "Hot Tub" folliculitis**



Skin and soft tissues infections

- ***Pseudomonas*** **dermatitis/folliculitis "Hot Tub" folliculitis**



Pseudomonas dermatitis/folliculitis

Exposure at a water slide

Salt Lake City, Utah

265 cases / 650

CDC, Morb. Mortal. Wkly.Rep. 1983;32:425-427).

Overgrowth of *Pseudomonas aeruginosa*

- Faulty maintenance of water in man-made pools
- Reduce the quantity of the bacterial organisms in the water
- Recommendations for treatment of pool water
  - maintaining the pH between 7.2 and 7.8
  - free-chlorine levels greater than 0.5 mg/liter.
- Some strains may be resistant to recommended chlorine concentrations (Khabbaz *et al.* , Am. J. Med. 1983;74:73-77).

Spas, whirlpools, hot tubes

More outbreaks than

Folliculitis

Folliculitis

Environment more conducive to the growth of micro-organisms

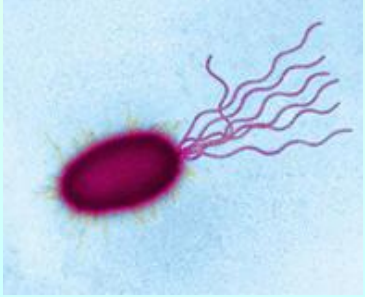
Difficulty in maintaining a stable free-chlorine levels

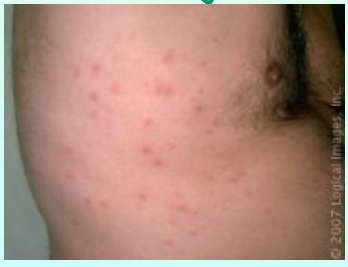
- higher temperature of the water
- mechanical agitation and aeration
- higher concentration of organic material (larger number of bathers per volume of water)

High water temperature

↓

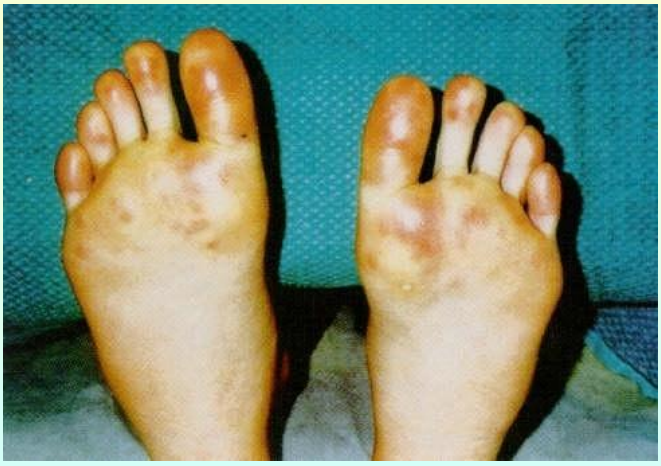
Dilatation of skin pores





Pseudomonas "hot-foot" syndrome

- Nodular lesions
- Soles of the feet



- Abrasive nature of the pool floor
  - Fiorillo *et al*. N. Engl. J. Med. 2001;345:335-338

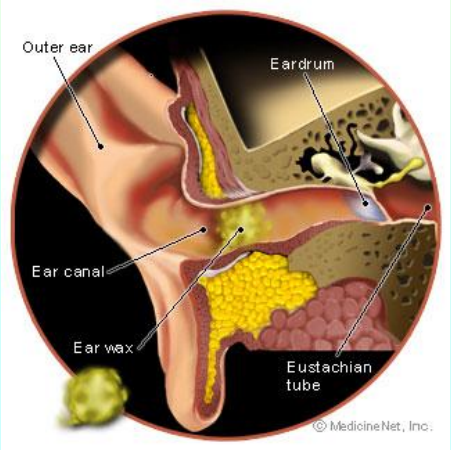
Acute diffuse otitis externa (swimmer's ear)

- More common in swimming pools users than whirlpool and spa users

- Water sport athletes (++): swimmers, divers, surfers, sailboarders, and kayakers in polluted bodies of water

Acute diffuse otitis externa (swimmer's ear)

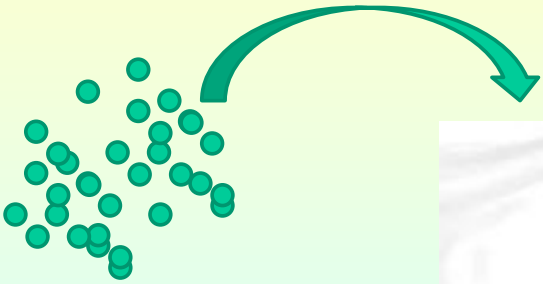
- Prolonged exposure to water causes maceration of the epithelial tissue in the ear canal and removes the ear wax



Aids: in repelling water
maintaining an acidic pH to prevent bacterial and fungal growth

P. aeruginosa pneumonia

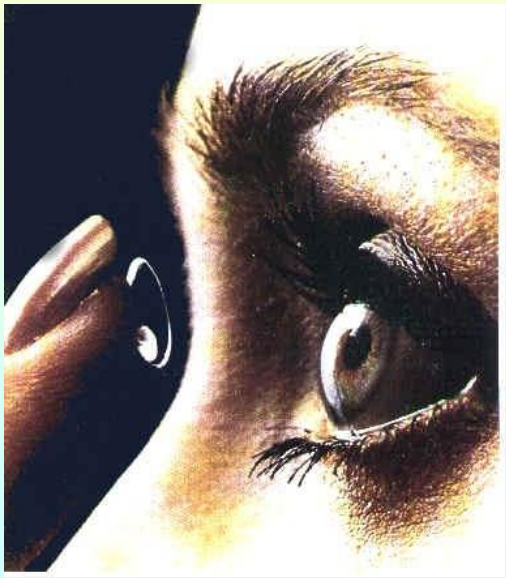
Whirlpool spa for 90 minutes



Rose *et al*. JAMA, 1983;250:2027-2029

P. aeruginosa keratitis

- Contact lens wearers

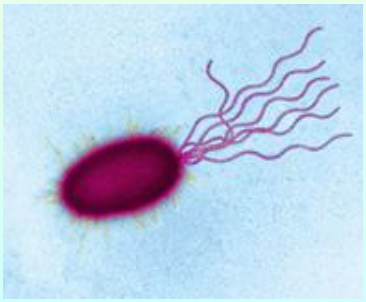


P. aeruginosa
a major hospital pathogen

- patients with compromised host defense mechanisms

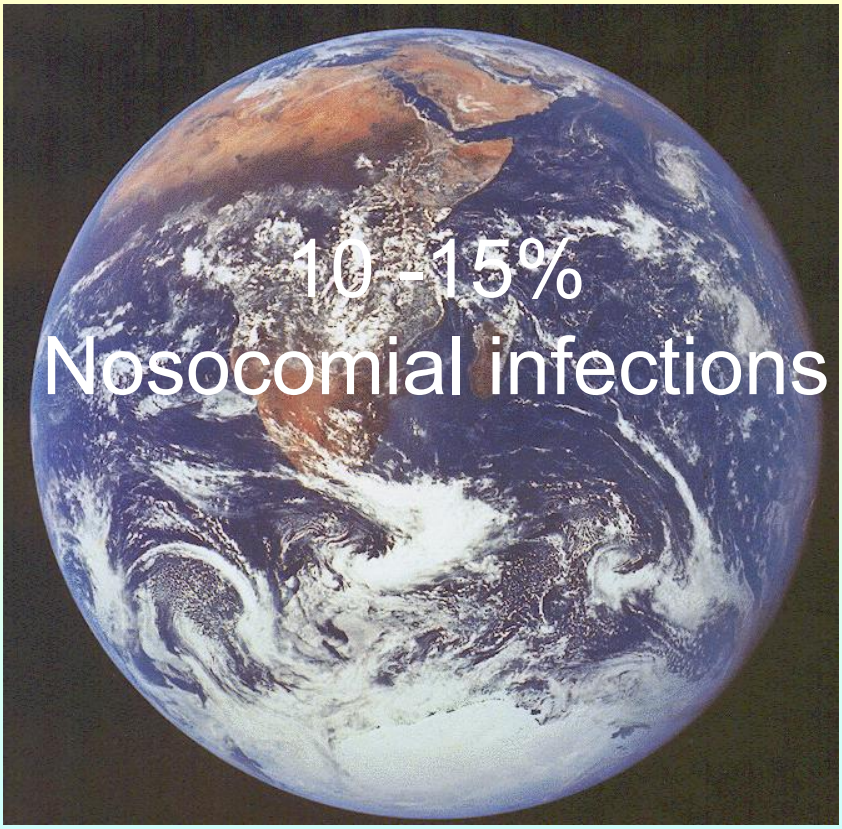
P. aeruginosa

- most common pathogen isolated from patients hospitalized longer than one week

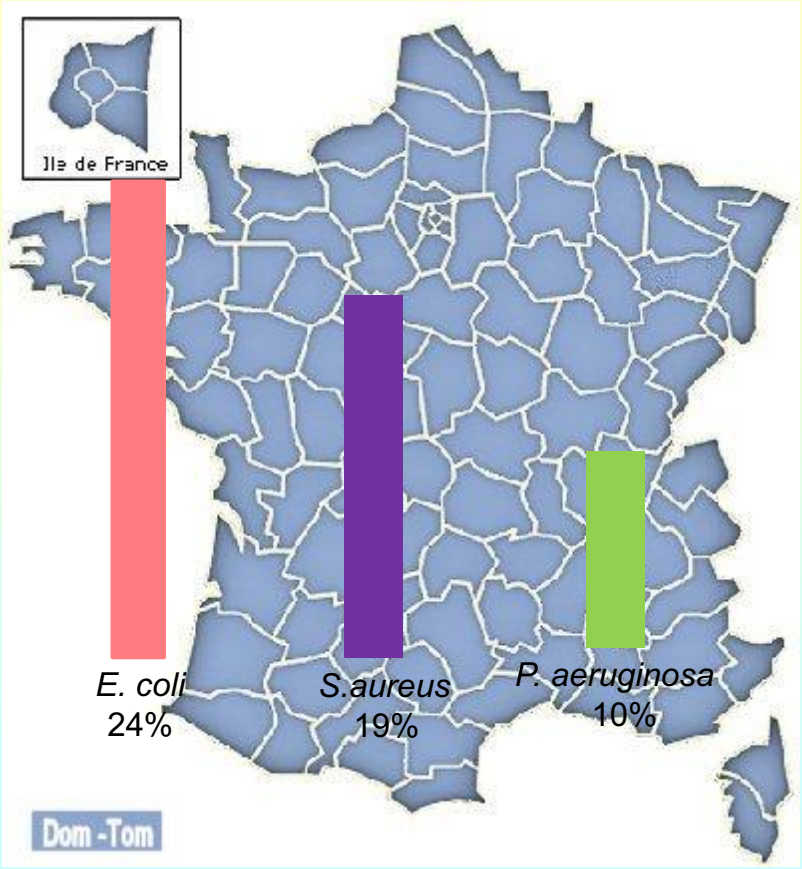


P. aeruginosa

Hospital Infections	Details and Common Associations	High-risk Groups
Pneumonia	Diffuse bronchopneumonia	Cystic fibrosis patients
Septic shock	Associated with skin lesion ecthyma gangrenosum	Neutropenic patients
Urinary tract infection	Urinary tract catheterization	
Gastrointestinal infection		
Necrotising enterocolitis (NEC)	NEC, especially in premature infants and neutropenic cancer patients	
Skin and soft tissue infections	Hemorrhage and necrosis	Burns victims and patients with wound infections



Pseudomonas aeruginosa



2007: National prevalence survey of nosocomial infections

P. aeruginosa & nosocomial infections

- Intensive care units (ICUs)(high endemic potential): 18 %

- vs

- Surgical and non-surgical units: 6%

  - Bertrand *et al*. Clin. Microbiol. Infect. 2001;7:706

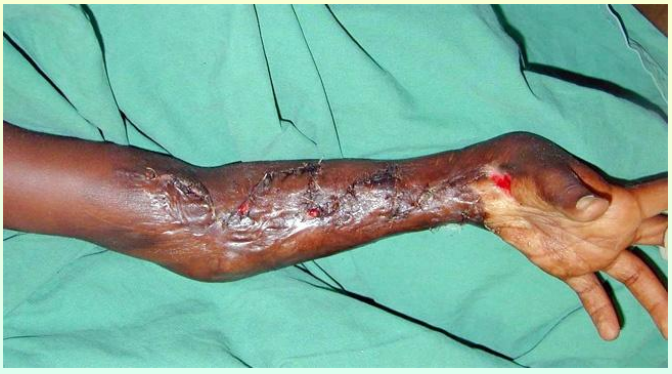
P. aeruginosa & nosocomial infections

- Mortality rates: 40% to more than 60%
  - Bacteraemic nosocomial pneumonia
  - Ventilator-associated pneumonia

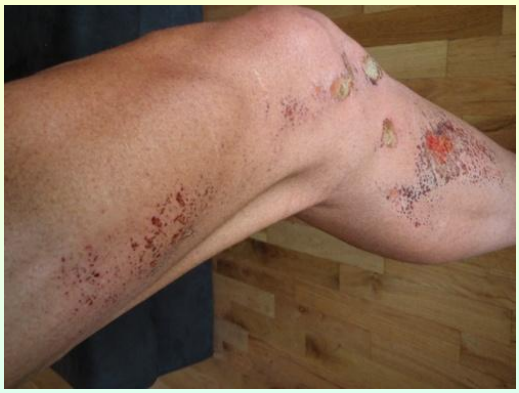
Recovery of *P. aeruginosa* in humans

2-10%

50-60%



Burns



Scabs

Environmental reservoirs of *P. aeruginosa* in hospitals
Potable water
Taps/sinks/sink traps
Showers
Disinfectants/sanitizers/antiseptics/bar soap
Respiratory therapy equipment
Ice makers
Flowers vases
Shaving/toothbrushes
Medication, e.g. eyes drops; multi-dose vials; mouthwash
Mop heads/buckets
Endoscope/endoscope washers
Urometers
Water baths
Hydrotherapy pools
Infant feeding basins
Bathing basins
Bath toys
Cleaning equipment

Medical and surgical equipments

Hospital fittings

Other material

Damp environments

- As a result, many hospital hygiene teams place great importance on the role of water in all infections with *P. aeruginosa*, especially in ICUs.

Intensive Care Med (2008) 34:1428–1433
DOI 10.1007/s00134-008-1110-z

ORIGINAL

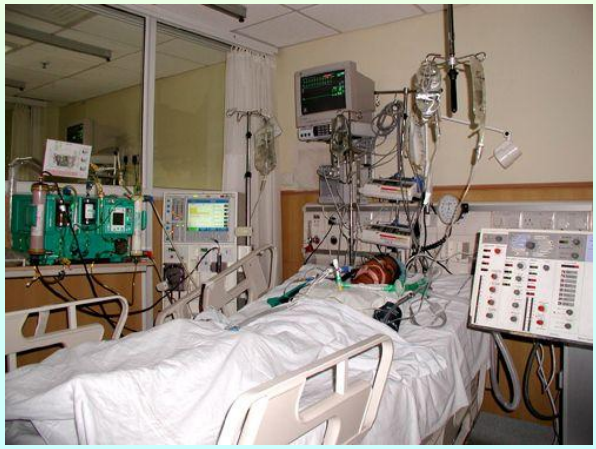
OpenAccess

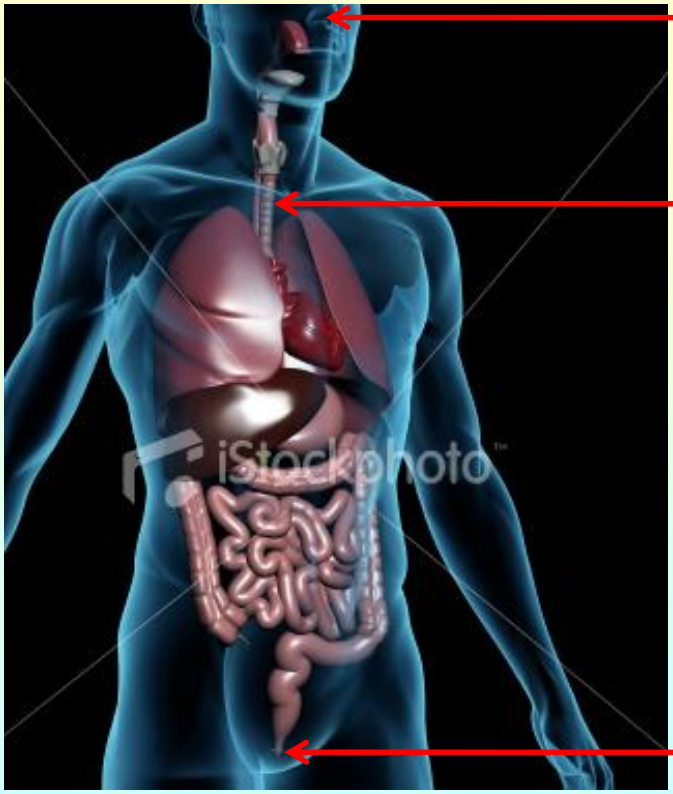
Pascal Cholley
Michelle Thouverez
Nathalie Floret
Xavier Bertrand
Daniel Talon

The role of water fittings in intensive care rooms as reservoirs for the colonization of patients with *Pseudomonas aeruginosa*

Clinical samples

- taken on admission of the patient in the ICU
- and one per week thereafter, throughout the patient's stay





- Colonization was defined as positive result for at least one sample

	Nose	Tracheal aspiration (TA)	Rectum	Nose + TA*	Nose + rectum*	TA + rectum*	Total (%)
Colonized patients (%)	21.9	32.2	27.4	13.7	4.1	0.7	100
Colonization at admission (%)	12.5	30	30	17.5	7.5	2.5	100
Colonization during hospitalization (%)	25.5	33	26.4	12.3	2.8	0	100

* : Simustanously positive samples

Distribution of first-positive screening sites upon admission and during hospitalisation in intensive care unit (X. Bertrand, personal comunication)

Incidence of colonization/infection

			Positive sample *P. aeruginosa*
ICU 123 patients	Medical unit	69	8
	Surgical unit	54	9

Overall incidence of colonization: 13.8 per 100 patients admitted

Environmental samples

- taken once per week from the water fittings in each ICU room

U-bend: 10 ml

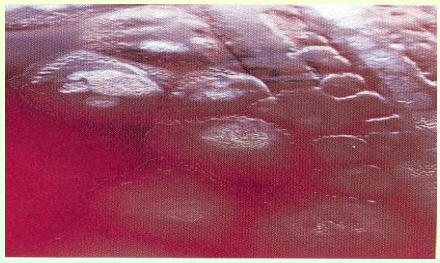
Tap water: 150 ml

Water environment

			Positive sample *P. aeruginosa*
ICU 448 samples	U-bends	224	193 (86.2%)
	Taps	224	10 (4.5%)

Pseudomonas aeruginosa

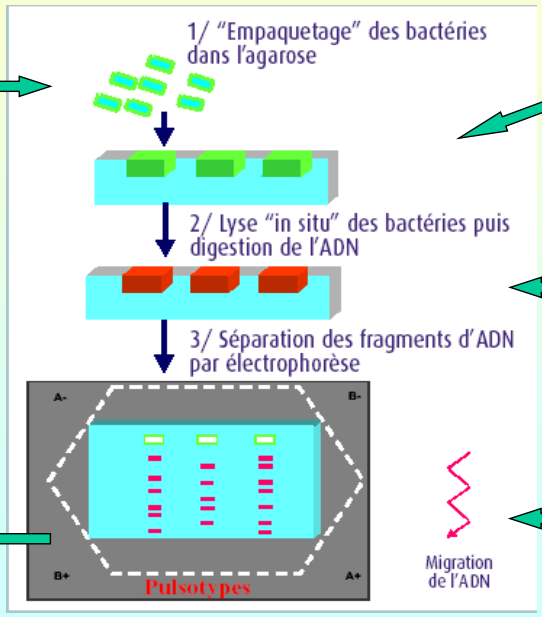
- **Identification**



Macrorestriction profile

Pulsed Field Gel Electrophoresis

1



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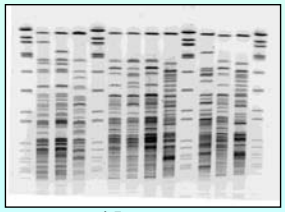

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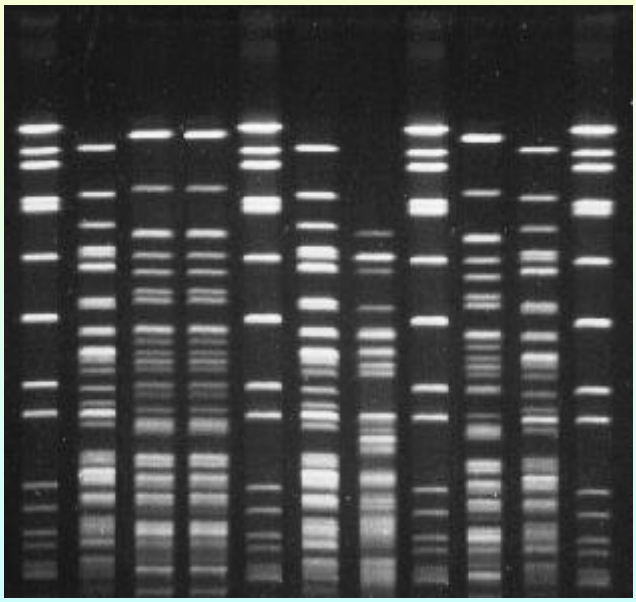
DNA macrorestriction profile



Molecular typing of clinical isolates

			Clinical isolates *P. aeruginosa*
ICU 123 patients	Medical unit	69	8
	Surgical unit	54	9

17 clones

Molecular typing of environmental isolates

<table>
<tr><td colspan="3">ICU</td></tr>
<tr><td rowspan="2">203 strains</td><td rowspan="2">82 pulsotypes</td><td>54 unique</td></tr>
<tr><td>28 multiple</td></tr>
</table>

- Only one patient was colonized with a clone present in the water environment of his room.

Pseudomonas aeruginosa

- The water environment played only a minor role in the colonization/contamination of patients

7.2%

U-bend: contamination +++

retro-colonization of the U-bend by the microflora present in wastewater pipes, via the biofilms

Role of the water environment on the colonization of patients hospitalized in ICU

Authors/year	Water samplings +	Patients +	% *
Ferroni/1998	21/118 (17,7%)	3/14	21,4
Berthelot/2001	34/NR	3/12	25
Trautmann/2000	49/72 (68%)	2/14	14,2
Reuter/2002	150/259 (57,9%)	5/17	29,4
Vallés/2004	93/149 (62,4%)	16/39	41,0
Blanc/2004	21/216 (10%)	36/132	27,3
Trautmann/2005	60/143 (41,9%)	8/16	50
Rogues /2007	65/673 (9,5%)	55/484	11,4
Cholley /2008	193/224 (86,2%)	1/14	7,1

*Patients colonized by a strain also found in the environment

Water fittings: colonization/infection of patients?

- Major role?
- Weak epidemiological link?
- Previous studies carried out during outbreaks

- The frequency of strains widely present in the environment (multiple clones) but never isolated from patients was high

Pseudomonas aeruginosa

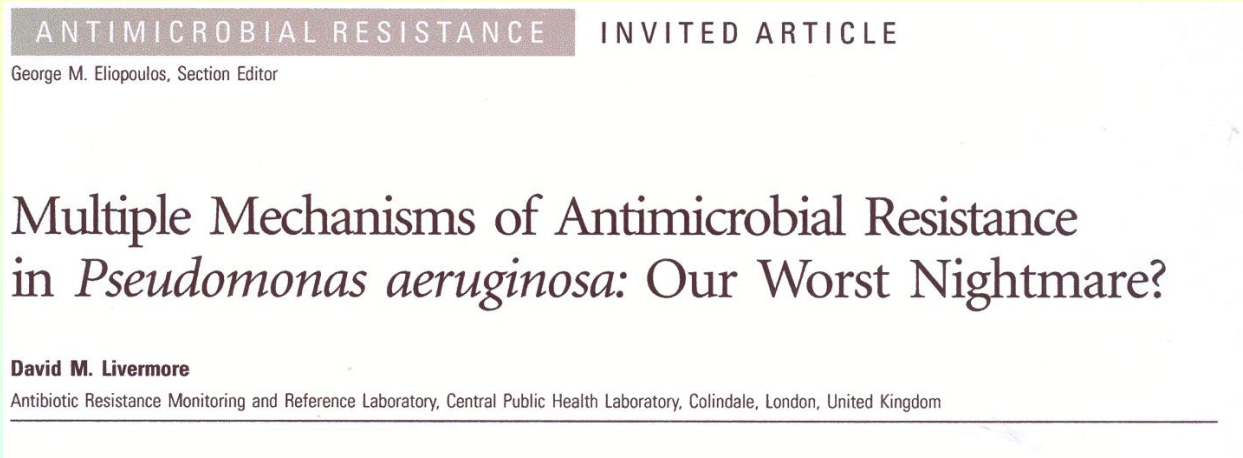
- There may be two different genetic groups:
  - one group of strains that are mostly environmental and not very pathogenic in humans

- Valles *et al*. Intensive Care Med. 2004;30:1768

Pseudomonas aeruginosa

- There may be two different genetic groups:
  - one group of strains that are mostly environmental and not very pathogenic in humans
  - one group of strains better adapted to humans with a much higher pathogenic potential

    - Valles *et al*. Intensive Care Med. 2004;30:1768



ANTIMICROBIAL RESISTANCE INVITED ARTICLE

George M. Eliopoulos, Section Editor

Multiple Mechanisms of Antimicrobial Resistance in *Pseudomonas aeruginosa:* Our Worst Nightmare?

David M. Livermore

Antibiotic Resistance Monitoring and Reference Laboratory, Central Public Health Laboratory, Colindale, London, United Kingdom

Antimicrobial resistant rates (%) of *P. aeruginosa* clinical isolates (EARSS: European Antimicrobial Resistance Surveillance System)

Country	Proportion (%) of strains non-susceptible to:				
	Aminoglycosides[a]	Carbapenems[b]	Quinolones[c]	Ceftazidime	Piperacillins[d]
Austria	11.2	13.7	17.9	9	7.1
Switzerland	4.8	5.4	7.2	4.2	5
Cyprus	25	21.1	21.2	15.4	28.8
Czech Republic	33.8	36	42.7	32.7	30
Germany	20.3	31.5	35.7	24.4	48.5
Denmark	2.4	3.9	9.1	4	4.8
Spain	23.9	18.4	27.7	15.2	8.1
Finland	8.7	9.4	10.9	7.7	7.3
France	31.1	18.4	26.3	18.6	20.5
Greece	51.9	50.5	51.9	44.8	38.4
Croatia	43.4	28.1	33	20.5	30.2
Hungary	34.4	21.3	29.5	15.3	16.8
Ireland	12.5	11.2	20.5	10.3	11.8
Israel	21.9	14.9	26.7	13.3	15.2
Italy	30.1	32.1	39.1	41.4	27.2
The Netherlands	9.8	5.4	9.4	5.6	5.2
Norway	1.9	14.5	10.7	6.7	3.1
Poland	40.3	22.4	40.3	22.7	35.8
Portugal	18.2	16.1	23	20.9	15.8
Sweden	0	9	10.3	9.6	3.1
Slovenia	13.6	20.4	18.1	13.6	12.5
Turkey	28.2	31	29.6	31.3	32.4
United Kingdom	6.6	17.2	9.6	14.1	5.4

Souli *et al*. Eurosurveillance, 2008;13, 1-11

Antimicrobial resistant rates (%) of *P. aeruginosa* clinical isolates in France (EARSS: European Antimicrobial Resistance Surveillance System)

Aminoglycosides	Carbapenems	Quinolones	Ceftazidime	Piperacillins Pip/Tazo
31.1	18.4	26.3	18.6	20.5

Souli *et al*. Eurosurveillance, 2008;13, 1-11

Antimicrobial resistance in *P. aeruginosa*

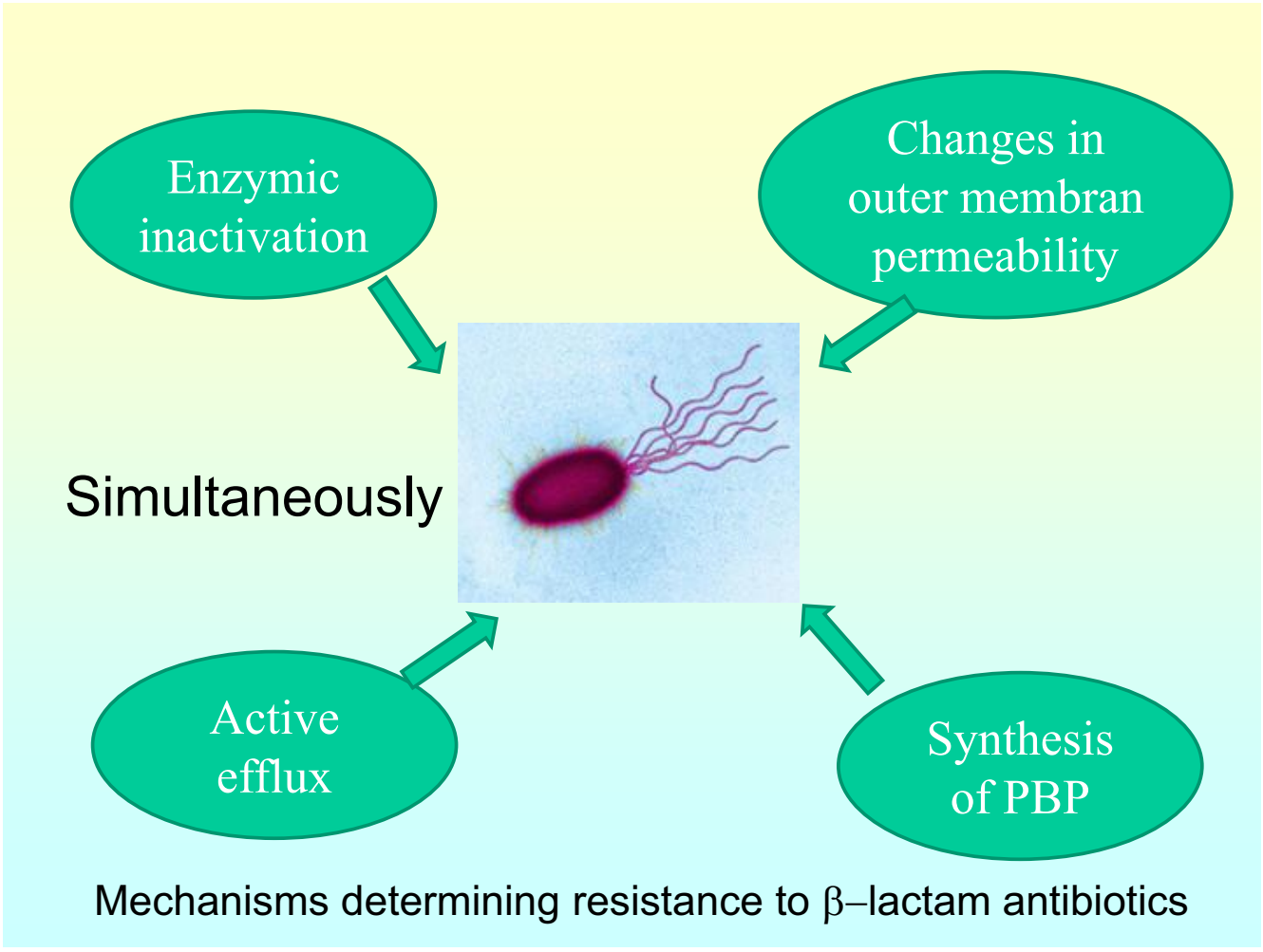
- low permeability of its outer membrane (>> Enterobacteriaceae)
  - 1% of the permeability of *E. coli* outer membran
- naturally occuring chromosomal AmpC cephalosporinase
- efflux resistance (mexAB-OprM)

P. aeruginosa wild type

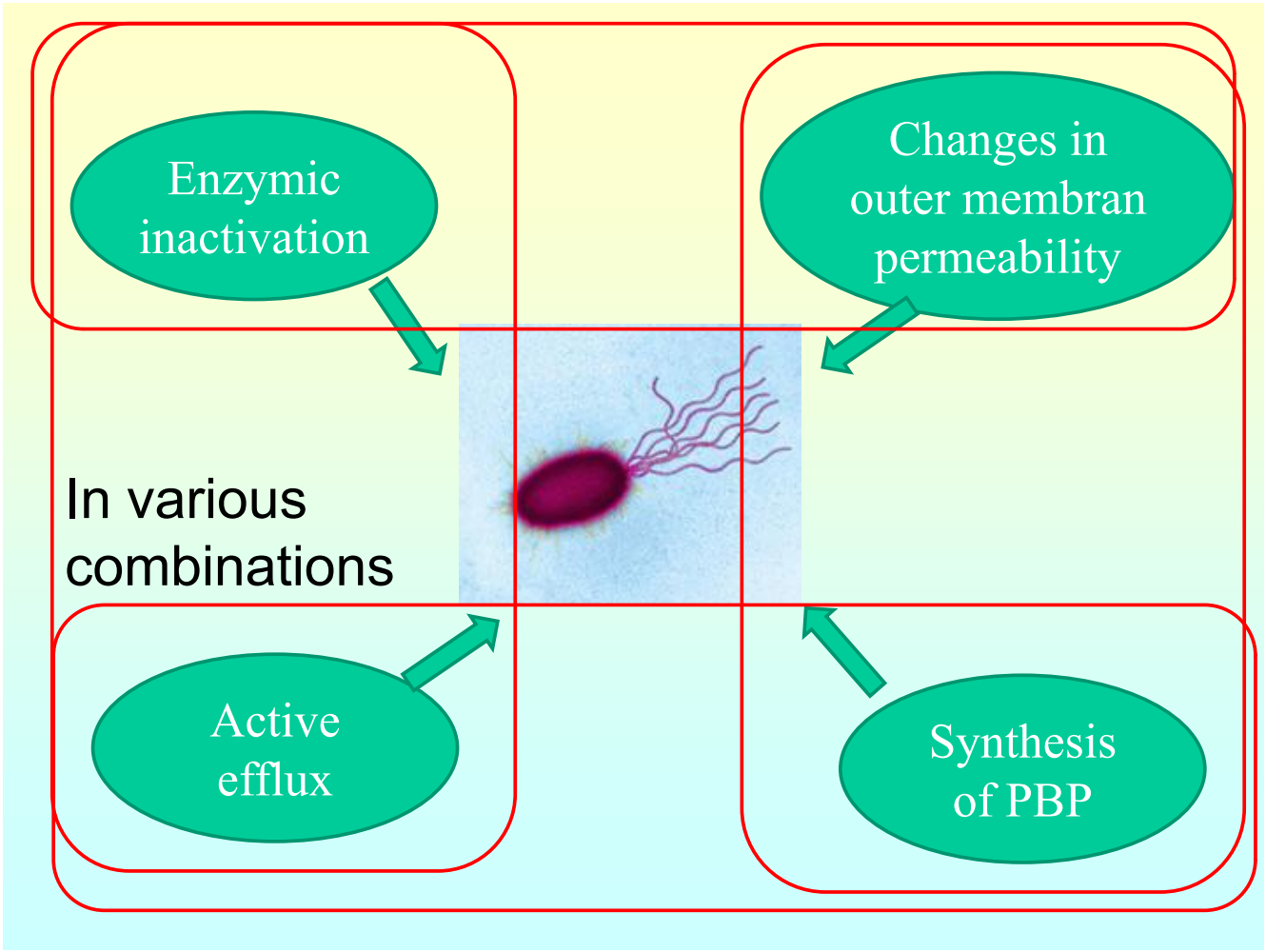
- Susceptible to
  - carboxypenicillins (carbenicillin, ticarcillin)
  - ureidopenicillins (azlocillin , piperacillin)
  - (some) third generation cephalosporins (ceftazidime, cefsulodine, cefoperazone)
  - fourth generation cephalosporins
  - monobactam aztreonam
  - carbapenems (imipenem, meropenem)

Antimicrobial resistance in *P. aeruginosa*

- Remarkable ability to acquire further resistance mechanisms to multiple groups of antimicrobial agents:
  - β-lactams
  - aminoglycosides
  - fluoroquinolones



Mechanisms determining resistance to β–lactam antibiotics



P. aeruginosa

- Enzyme production is the major mechanism of acquired resistance to β-lactam antibiotics

Resistance to β-lactams due to β-lactamase production (1)

		Resistance to	Comment
AmpC β-lactamase (not inhibited by β -lactamase inhibitors)	"low level expression"	Aminopenicillins ~~Most of early cephalosporins~~	Chromosomal
	Hyperproduction	Third generation cephalosporins	++
Class A carbenicillin hydrolyzing β-lactamase (PSE: *Pseudomonas* specific enzyme:)(PSE-1, PSE-4, CARB-3, CARB-3)		Carboxypenicillins Ureidopenicillins Cefsulodine	
Class A ESBLs (SHV, TEM, VEB, PER, GES, IBC, BEL-types)		Carboxypenicillins Ureidopenicillins Extended-spectrum cephalosporins (ceftazidime, cefepime, cefpirome) Aztreonam	In vitro inhibition by clavulanic acid & tazobactam Chromosomal & plasmid

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Resistance to β-lactams due to β-lactamase production (2)

		Resistance to	Comment
Class D β-lactamase (OXA: oxacillinases)	Classical OXA enzymes (OXA-1, OXA-2, OXA-10)	Carboxypenicillins Ureidopenicillins Not to ceftazidime	
	Ceftazidime hydrolyzing extended-spectrum oxacillinases	Ceftazidime, cefotaxime,cefepime, cefpirome, aztreonam and moxalactam	Not suppressed by clavulanic acid & tazobactam (except OXA-18)
Class B MBLs* (IMP-type, VIM-type, SPM-1, GIM-1)	Carbapenemases (Carbapenem hydrolyzing enzymes)	All β-lactams including the carbapenems (imipenem, meropenem)	Non inhibited by clavulanic acid & tazobactam Monobactam aztreonam not influenced

*metallo-β-lactamase

Resistance to β-lactams due to β-lactamase production (2)

		Resistance to	Comment
Class D β-lactamase (OXA: oxacillinases)	Classical OXA enzymes (OXA-1, OXA-2, OXA-10)	Carboxypenicillins Ureidopenicillins Not to ceftazidime	
	Ceftazidime hydrolyzing extended-spectrum oxacillinases	Ceftazidime, cefotaxime,cefepime, cefpirome, aztreonam and moxalactam	Not suppressed by clavulanic acid & tazobactam (except OXA-18)
Class B MBLs* (IMP-type, VIM-type, SPM-1, GIM-1)	Carbapenemases (Carbapenem hydrolyzing enzymes)	All β-lactams including the carbapenems (imipenem, meropenem)	Non inhibited by clavulanic acid & tazobactam Monobactam aztreonam not influenced

*metallo-β-lactamase

Resistance to β-lactams due to active efflux

- *P. aeruginosa* less susceptible than *Enterobacteriaceae* to antibiotics
- Low outer membran permeability (proteins with high molecular mass)
- Proteins (OprM, OprJ, OprN) act as components of active efflux systems

- Resistance determined by interplay between:
  - low membrane permeability
  - efflux of antimicrobial agents

Structure and substrate specificity of the three-component active efflux systems in *P. aeruginosa*

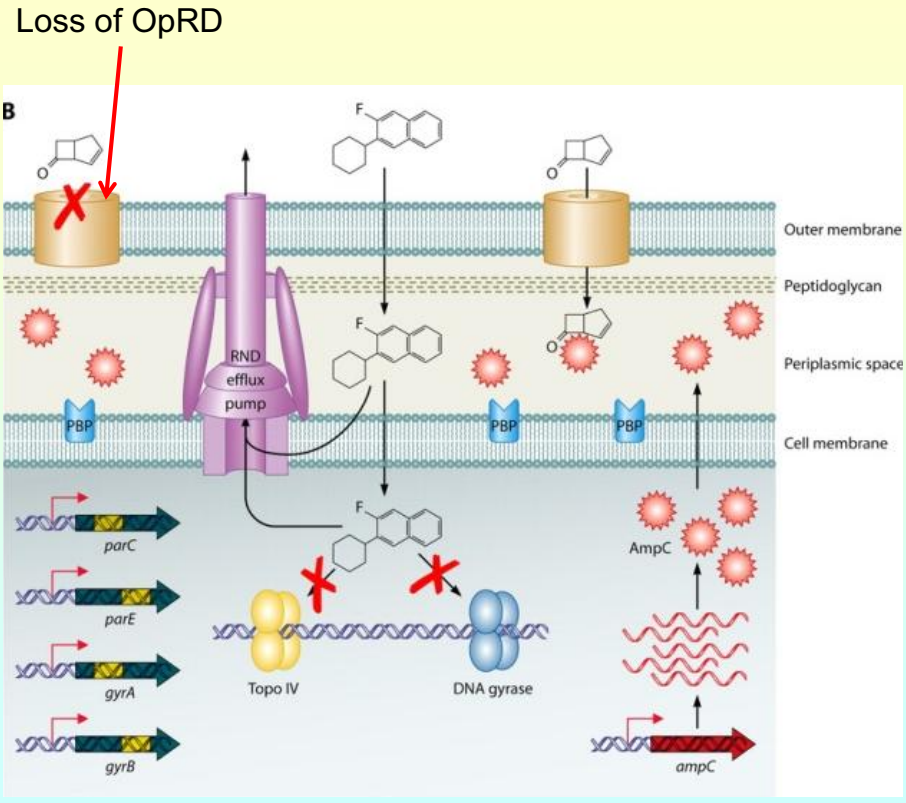
Cytoplasmic Membrane pump	Periplasmic linker	Outer membrane channel	Substrate
MexB	MexA	OprMp	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, β-lactams except imipenem
MexD	MexC	OprJ	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, penicillins except carbenicillin and sulbenicillin, cefepime, cefpirome, meropenem
MexF	MexE	OprN	Fluoroquinolones, carbapenems
MexY	MexX	OprM	Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, aminoglycosides, penicillins except carbenicillin and sulbenicillin, cefepime, cefpirome, meropenem

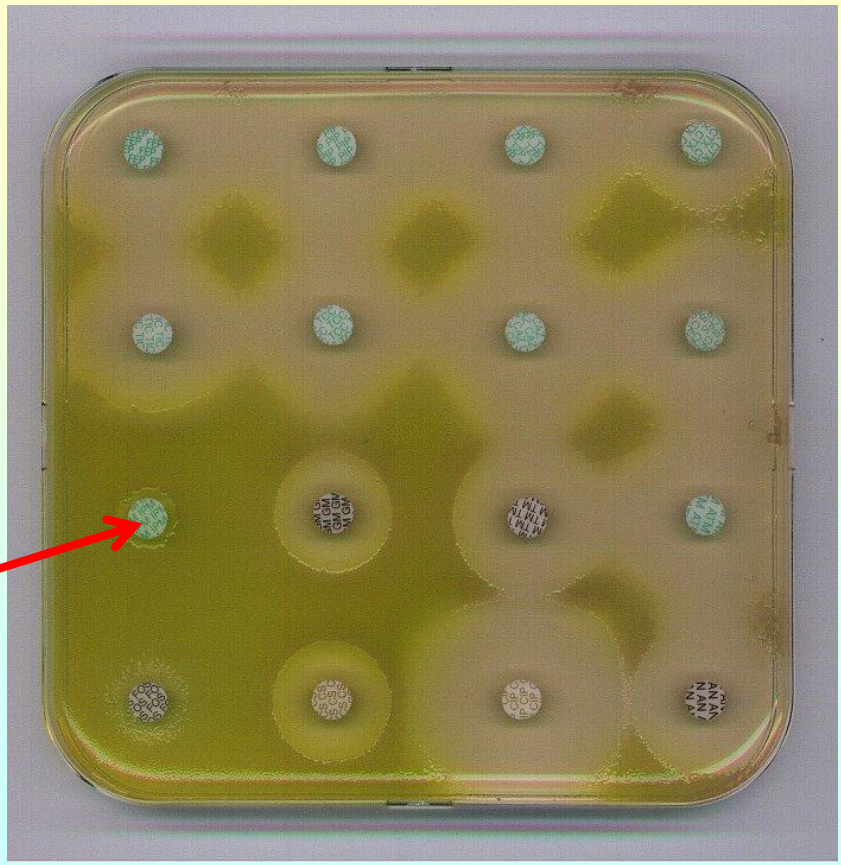
Resistance to β-lactams due to altered outer membrane permeability

- Imipenem-resistant *P. aeruginosa*: deficiency of OprD (referred to as D2 porin)

only in case of expressed chromosomal AmpC β-lactamase

close cooperation between these two mechanisms



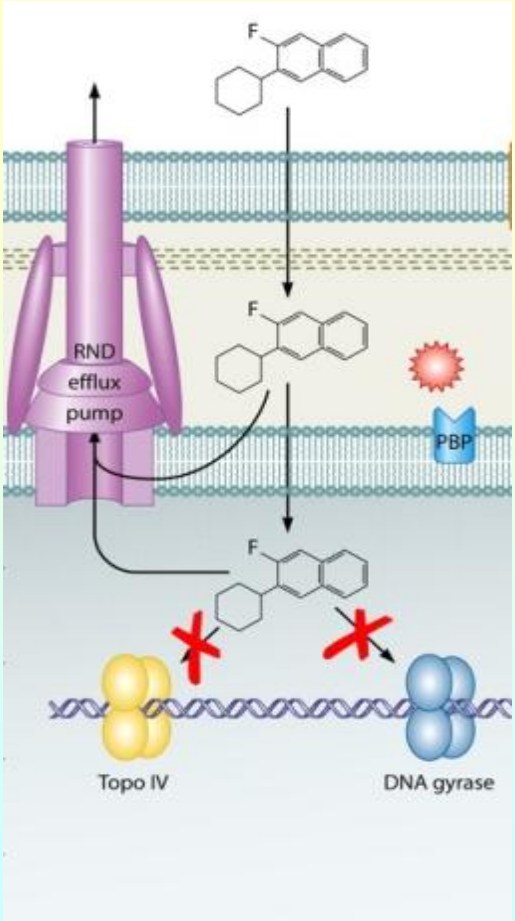


Mechanisms of resistance to aminoglycosides

- Enzyme modification (major)
- Low outer membrane permeability
- Active efflux
- Target modification (rarely)

Mechanisms of resistance to fluoroquinolones

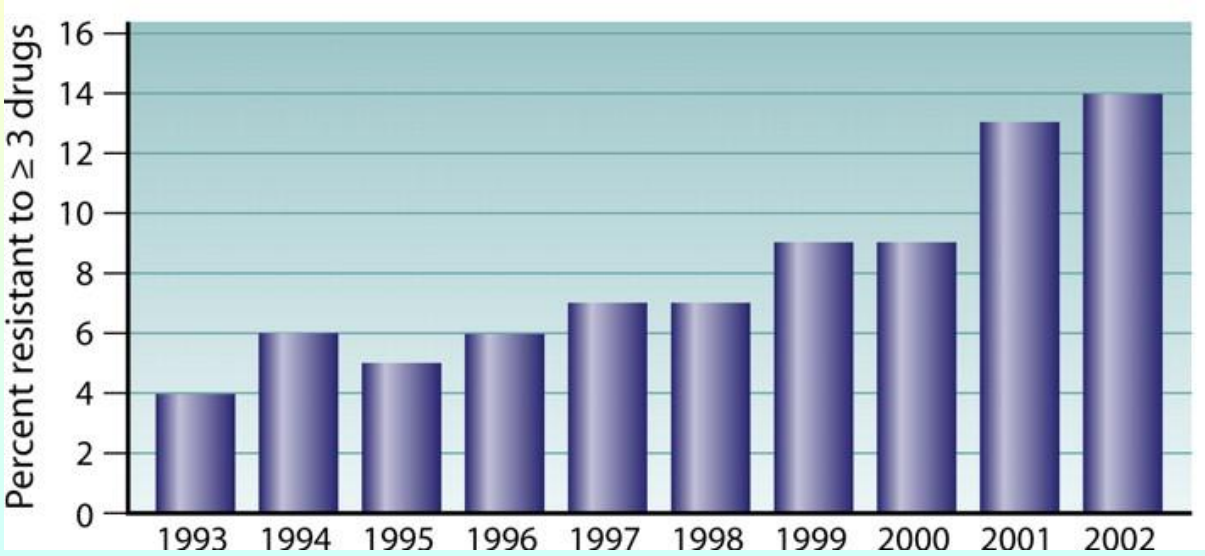
- Structural changes in target enzymes
  - DNA gyrase (or topoisomerase II): point mutations in *gyrA/gyrb* genes → low binding affinity to quinolone molecules
- Active efflux



Incidence of acquired-MDR

- *P. aeruginosa*: 0.1/1000 patient-days
- MRSA : 0.275/1000 patient-days
- ESBL-producing *Enterobacteriaceae*: 0.263/ 1000 patient-days

- Prevalence of MDR-PA: 10-15%



Prevalence of multidrug resistance among *P. aeruginosa* isolates from ICU patients in the USA (Lister *et a*l. Clin. Microbiol. Rev. 2009;22:582-610)

Journal of Hospital Infection 76 (2010) 316–319

Available online at www.sciencedirect.com

Journal of Hospital Infection

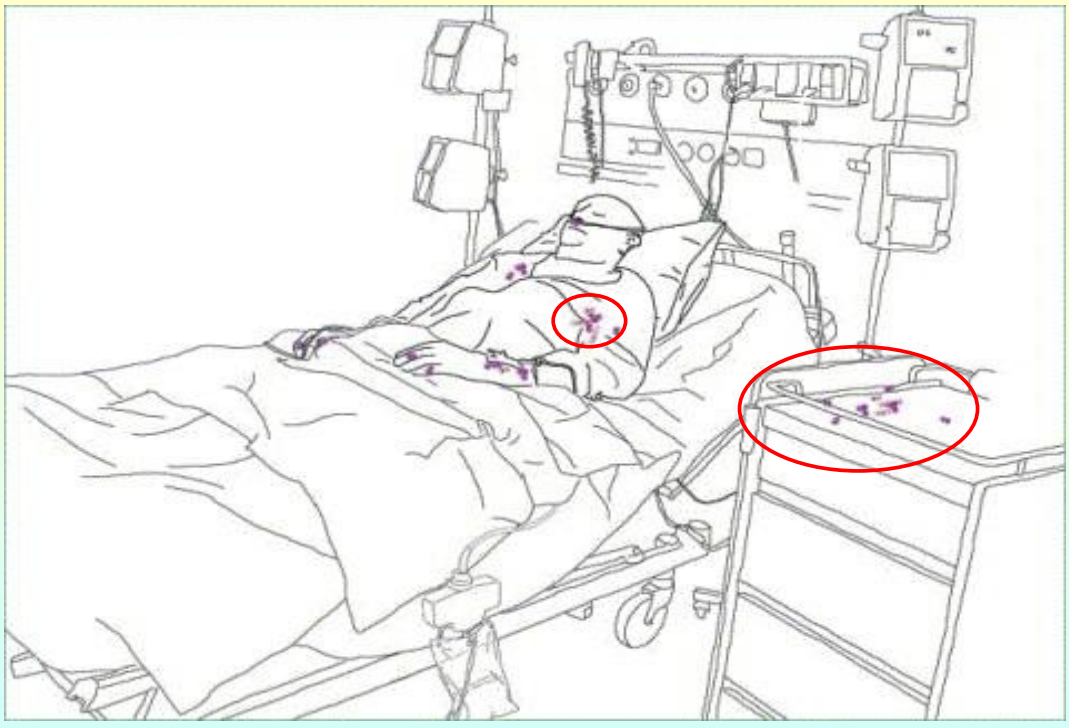
journal homepage: www.elsevierhealth.com/journals/jhin

Molecular epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in a French university hospital

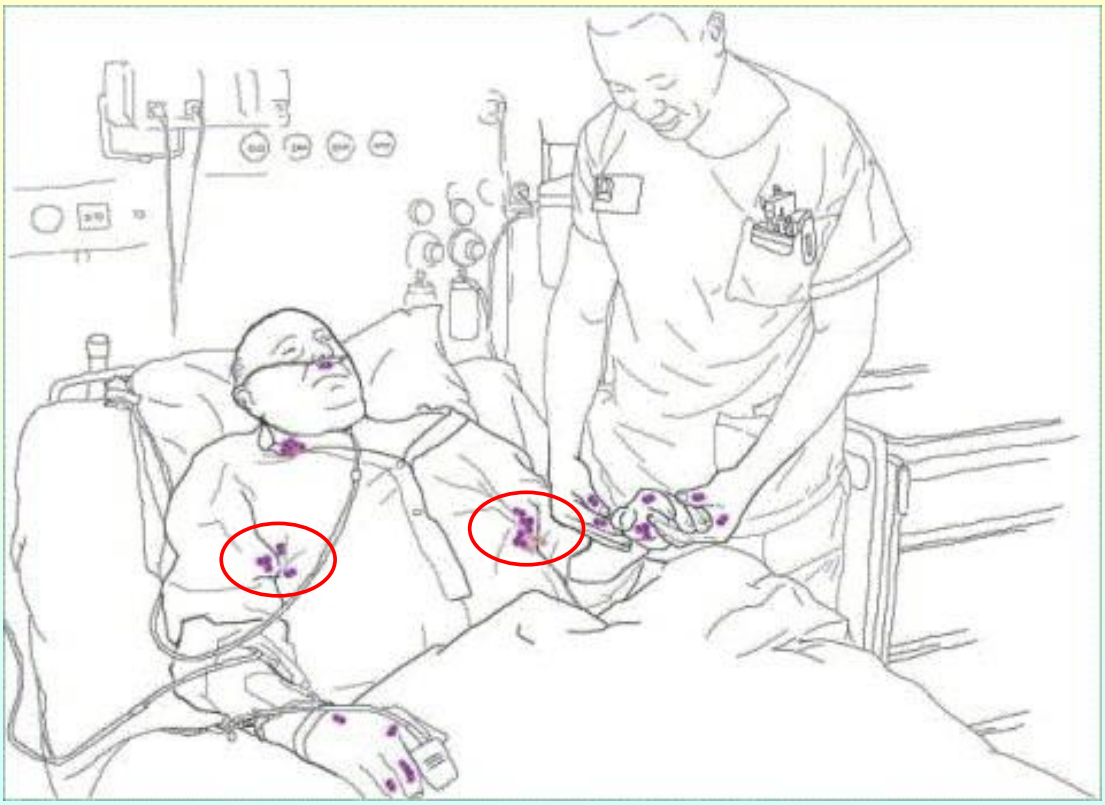
P. Cholley[a,b], H. Gbaguidi-Haore[a,b], X. Bertrand[a,b,c], M. Thouverez[a,b], P. Plésiat[c,d], D. Hocquet[c,d], D. Talon[a,b,c,*]

	MDR-PA		
Patients		Within 48 h of admission	After 48 h (mean period 41 days)
654/60,454	38 (5.8%)	2	36
	12 different PFGE patterns (A to L)		

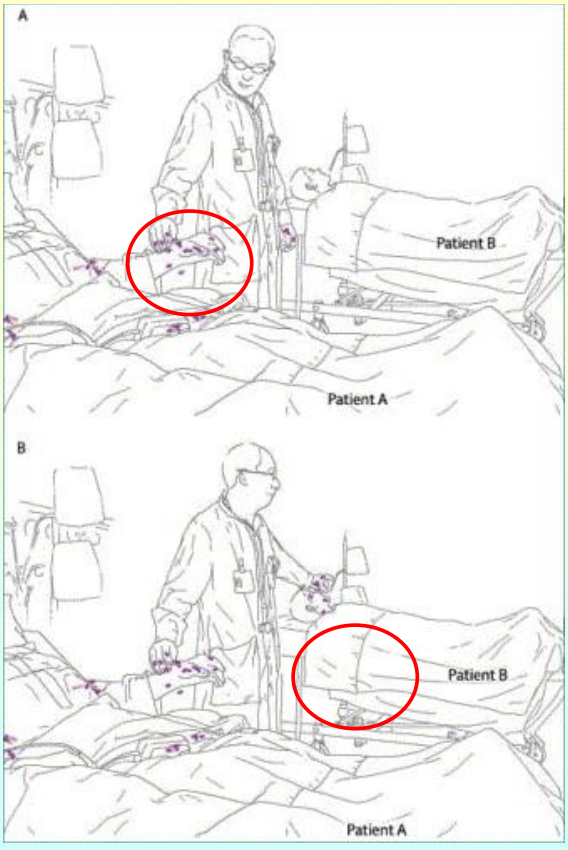
- Genotyping showed that cross-transmission was responsible for 70% of MDR *P. aeruginosa* cases



Microbes which are on the patient are able to spread the infection



Personnel in contact with these patients were the source…



of transmission of the micro-organisms to susceptible patients.

- Priority should be given to the improvement of standard hygienic precautions
- Antimicrobial rotation
- Restriction of certain agents

Consensus

- *P. aeruginosa* population is nonclonal epidemic
- clinical isolates are not distinguishable from environmental isolates
- no specific clones with a specific habitat or disease

  - Pirnay *et al*. PLoS One. 2009;4:e7740

- The majority of multidruresistant *P. aeruginosa* isolates from hospitals belongs to a few clonal types
    - Cholley *et al*. , 2010 (in press)

Multilocus sequence typing (MLST)

- Typing of multiple loci
- DNA sequences of internal fragments of multiple housekeeping genes
- 450-500 bp internal fragments of each gene used
- allelic profile or sequence type (ST)

Multilocus sequence typing (MLST)

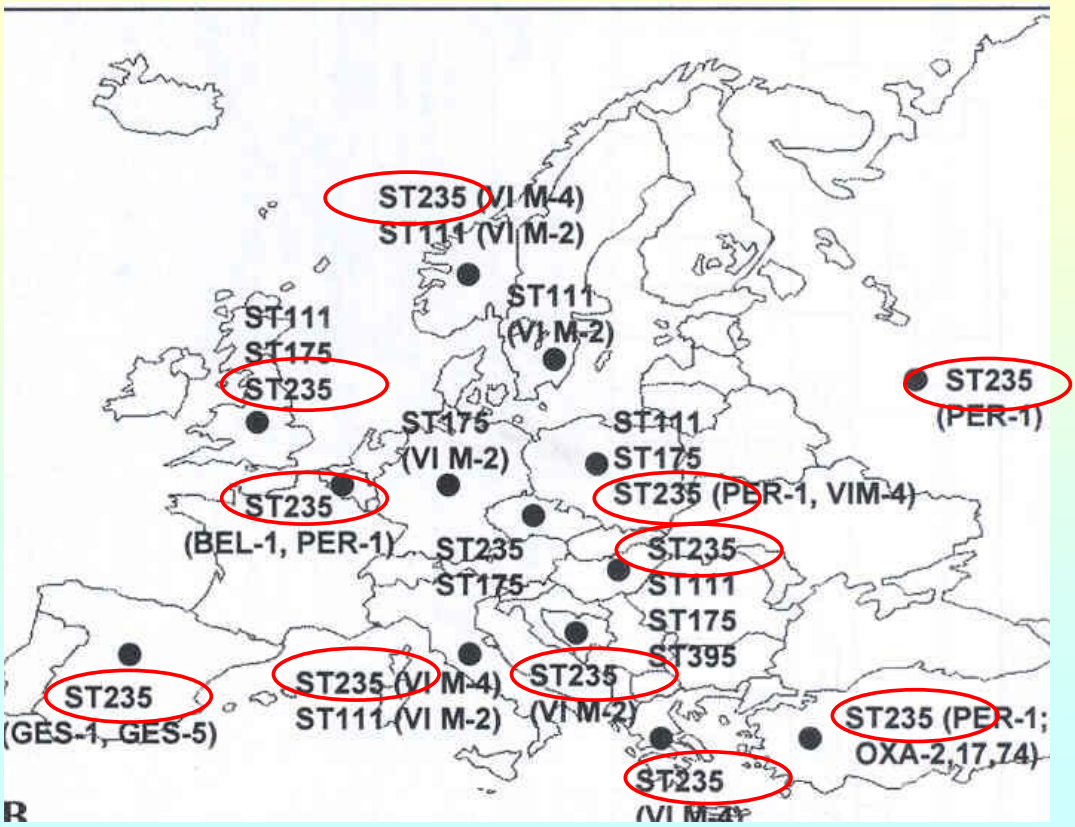
- accumulation of nucleotide changes in housekeeping genes:
  - relatively slow process
  - stable over time
  - global epidemiology

MLST

- 187 MDR-PA isolates
- Nucleotides sequences were determined for internal fragments of the
  - *acsA, aroE, guaA, mitL, nuoD, ppsA* and *trpE* genes

MLST

- The majority of MDR isolates belongs to a few clonal types: ST235, ST111 and ST175
- ST 235 was the founder of the clonal complex CC235 (internationally distributed and already attributed to various ESBL)



- The most successful clones are also more likely to acquire MDR determinants

Journal of Hospital Infection (2009) 73, 338–344

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REVIEW

Pseudomonas aeruginosa: a formidable and ever-present adversary

K.G. Kerr [a,*], A.M. Snelling [b]

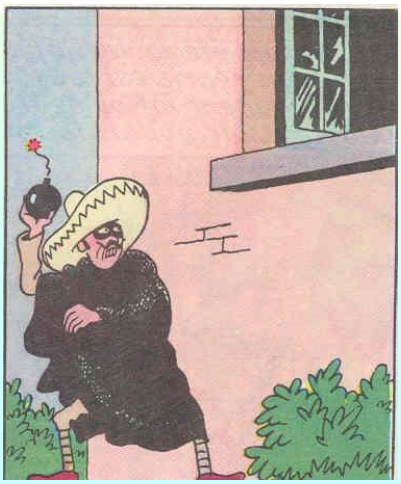
ANTIMICROBIAL RESISTANCE INVITED ARTICLE

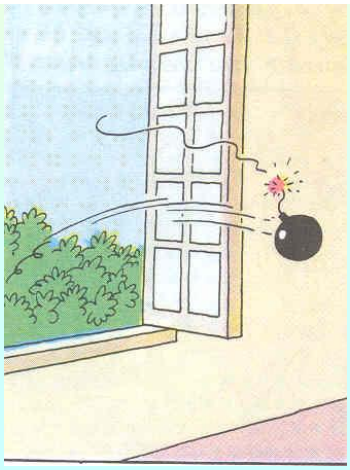
George M. Eliopoulos, Section Editor

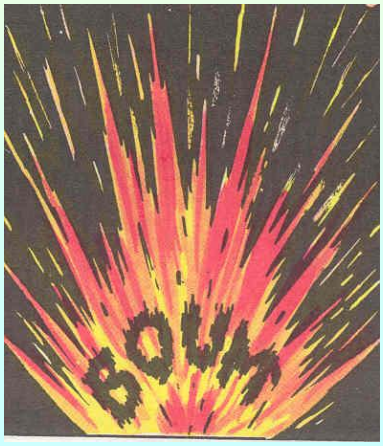
Multiple Mechanisms of Antimicrobial Resistance in *Pseudomonas aeruginosa:* Our Worst Nightmare?

David M. Livermore

Antibiotic Resistance Monitoring and Reference Laboratory, Central Public Health Laboratory, Colindale, London, United Kingdom







- Many thanks to Professor Xavier Bertrand and to the *Pseudomonas aeruginosa* team of Besançon