World Congress and Exhibition on

Antibiotics

September 14-16, 2015 Las Vegas, USA

OMICS International
conferenceseries.com

The First Report of CMY, AAC(6')-Ib and 16S rRNA Methylase Genes among *Pseudomonas aeruginosa* Isolates from Iran

Sedigheh Rafiei Tabatabaei, MD, MPH
Associate Professor of Pediatric Infectious Diseases
Pediatric Infections Research Center (PIRC),
Mofid Children's Hospital
Tehran, Iran

Shahid Beheshti
University of Medical Sciences

Co-Researchers

- Abdollah Karimi[1];
- Mohsen Jafari[1] ;
- Fatemeh Fallah[1] ;
- Rebwar Shams Borhan[2] ;
- Masoumeh Navidinia[1] ;
- Farideh Shiva[1];
- Ali Hashemi [2]

1. *Pediatric Infections Research Center, Shahid Beheshti University of Medical Sciences,*
2. *Department of Microbiology, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran*

What is The Problem?

THREAT OF ANTIBIOTIC RESISTANCE

DEVELOPING NEW ANTIBIOTICS

If bacteria do not respond to the drugs designed to kill them, we return to the times when simple infections were often fatal.

Background

For > 60 yrs, antibacterial drugs are regarded as the panacea to cure infections, whether or not their use is appropriate,

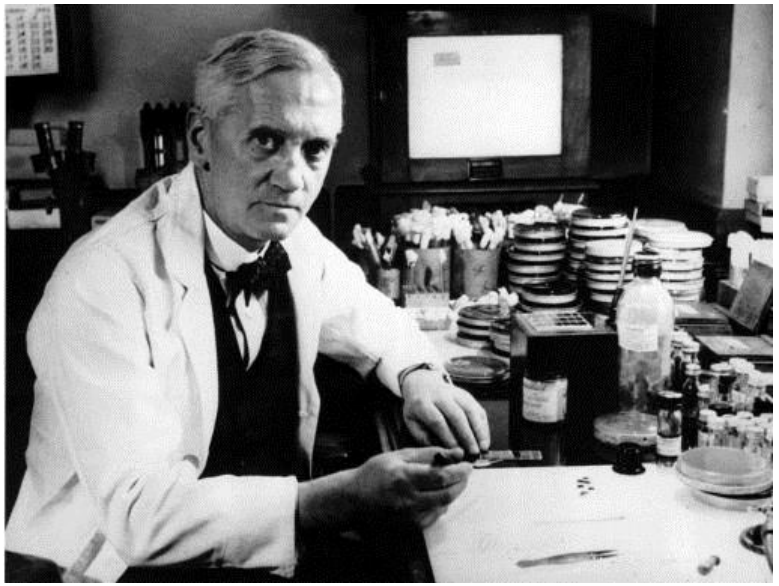
Development of resistance is a normal evolutionary process for microorganisms, but it is accelerated by selective pressure by widespread use of antibacterials.

ABR results in reduced efficacy of antibacterials, making treatment of patients difficult, costly, or even impossible.

Miracle Drug?

When penicillin - the first antibiotic - was discovered in 1928, it flipped medicine on its head ... in a good way.

It was called a "**miracle drug**," suddenly offering a cure for some of the most nefarious of diseases.



Sir Alexander Fleming, 1952

Superbug?

- It's a term coined by the media to describe bacteria that cannot be killed using multiple antibiotics.

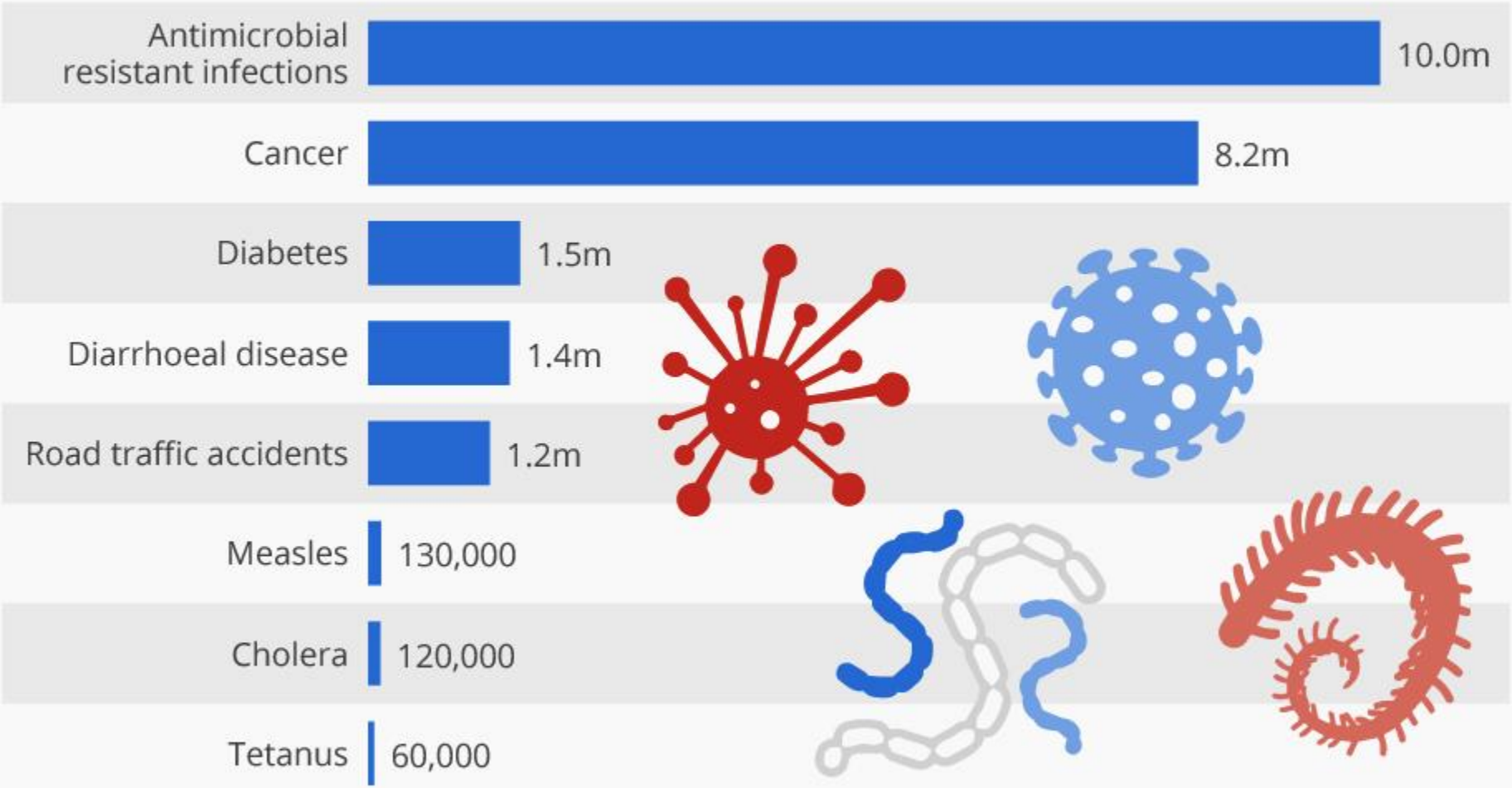
- "It resonates because it's scary," - Stephen Calderwood, president of the IDSA.

Should we be worried?

The WHO has identified antibiotic resistance as one of the greatest threats to human health today.

Deaths From Drug-Resistant Infections Set To Skyrocket

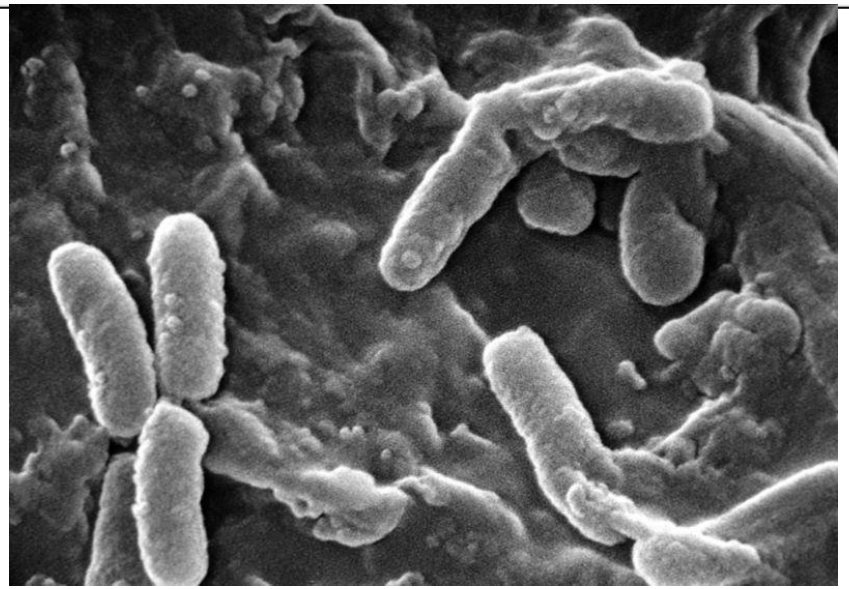
Deaths from antimicrobial resistant infections and other causes in 2050



The Review on Antimicrobial Resistance, Chaired by Jim O'Neill, December 2014

P.aeruginosa

- Often resistant to multiple antibiotics & consequently has joined the ranks of '**superbugs**' due to its enormous capacity to engender resistance.



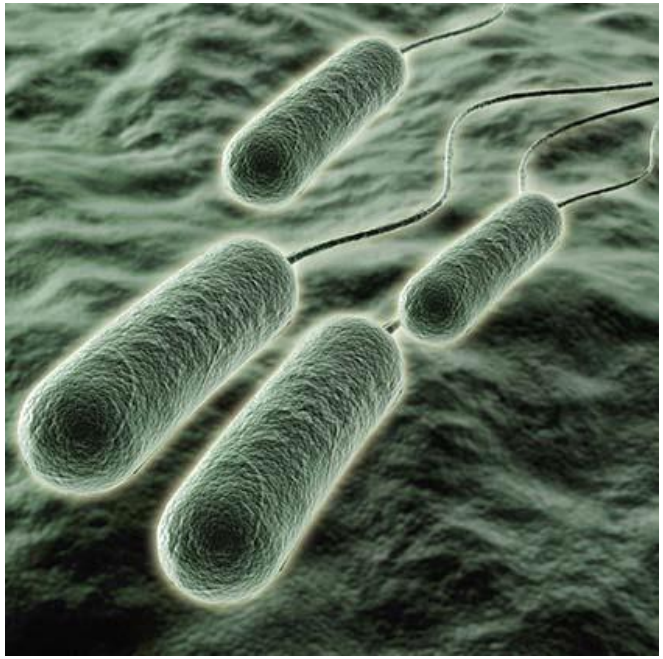
P. aeruginosa

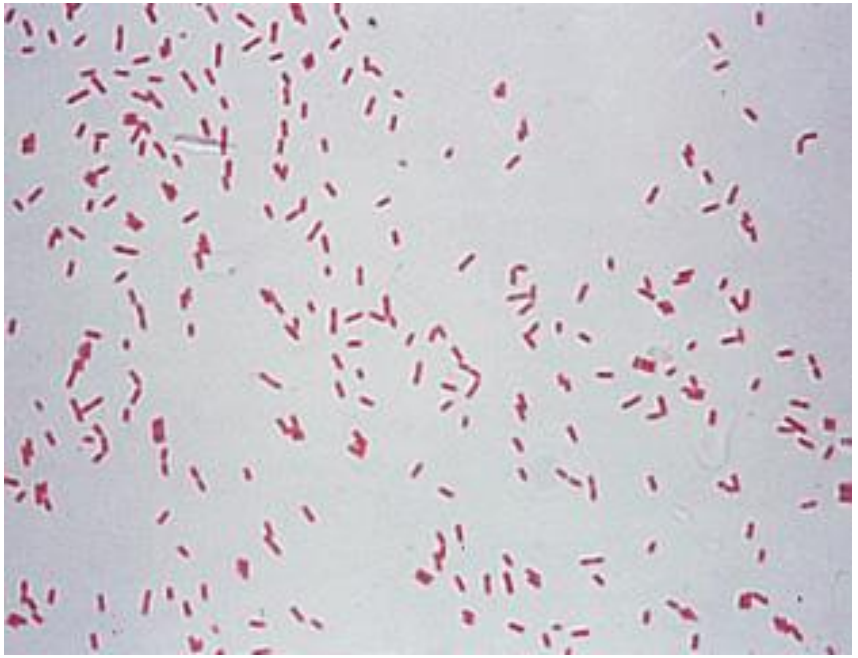
P. aeruginosa is a common cause of HCAIs including pneumonia, BSIs, UTIs, and SSIs.

Some strains of *P. aeruginosa* have been found to be resistant to nearly all or all antibiotics including AGs, CPs, FQs, and carbapenems.

Meaning several classes of antibiotics no longer cure these infections.

Microbiology





P. aeruginosa is a Gram-negative opportunistic pathogen that is commonly associated with HCAIs.

Despite the wide distribution of *Pseudomonas* in the environment, this microorganism rarely colonizes humans.

The pathogenic potential of *Pseudomonas* manifest in situations in which the host's immune defenses are diminished or lacking.

The organism generally does not invade intact skin or mucous membranes.

The most important species that causes human disease is ***P. aeruginosa***.

At risk patients

- Poor mucociliary clearance (CF),
- Neutropenia (secondary to cancer chemotherapy),
- Damaged skin barrier (burn wound),
- Immunodeficiency,
- Malnourished,
- Receiving immunosuppressive therapy,
- Having indwelling devices.

Burn Wounds

- Burn wounds & skin grafts - frequently colonized by *pseudomonads* & other gram-negative organisms.
- *MRSA* and *P. areuginosa* - most prevalent infectious agents.
- Burn wound infection - delays healing, encourages scarring, may result in bacteremia, sepsis or MODS.
- *P. aeruginosa* sepsis in burn patients has a mortality rates of up to **78%**.

Some Risk factors for Systemic Infections

- Prolonged use of IV / urinary catheters
- Antibiotics for prophylaxis – kill susceptible microbiologic flora but select for resistant strains of *P. aeruginosa*.
- Hydrotherapy

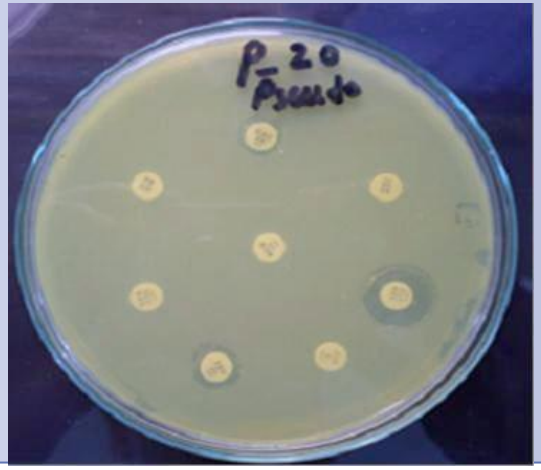
Burn hospitals often harbor MDR P. aeruginosa –a source of infection.

MDR *P. aeruginosa*

Resistance to at least 3 classes of currently available antimicrobials (Wang et al., 2006)

XDR

PDR



A pan-drug resistant isolate of Pseudomonas aeruginosa from a burn patient.

P.aeruginosa

Examples of antimicrobial susceptibility profiles that fit MDR, XDR & PDR definitions

Antimicrobial category	Antimicrobial agent	Isolate no. 1 (PDR)	Isolate no. 2 (XDR)	Isolate no. 3 (MDR)
Aminoglycosides	Gentamicin	X	X	
	Tobramycin	X		
	Amikacin	X		
	Netilmicin	X		
Antipseudomonal carbapenems	Imipenem	X	X	X
	Meropenem	X	X	
	Doripenem	X	X	
Antipseudomonal cephalosporins	Ceftazidime	X		X
	Cefepime	X	X	
Antipseudomonal fluoroquinolones	Ciprofloxacin	X	X	X
	Levofloxacin	X		
Antipseudomonal penicillins + β-lactamase inhibitors	Piperacillin-tazobactam	X		
	Ticarcillin-clavulanic acid	X	X	
Monobactams	Aztreonam	X	X	
Phosphonic acids	Fosfomycin	X		
Polymyxins	Colistin	X		
	Polymyxin B	X		

OBJECTIVE

To determine the prevalence of

CMY, AAC (6′)-Ib &16S rRNA methylase genes among *P. aeruginosa* isolates from burn patients,

PATIENTS & METHODS

Isolation and Clinical Identification

From September 2011 to January 2012,

448 burn patients

level I Burn Care center in Tehran

100 isolates of *P. aeruginosa* were collected by sterile swabs

Isolation

P. aeruginosa

is readily recovered from clinical specimens and usually is recognized easily on laboratory media.

Antibiogram of *P. aeruginosa* on Mueller Hinton Agar

Methods…

Antimicrobial Susceptibility Testing (CLSI guideline):

- **Disk Diffusion** (Merck, Germany)
- **MIC (**broth microdilution method)

Zone Diameter and MIC Interpretive Standards for *P. aeruginosa*- CLSI guidelines

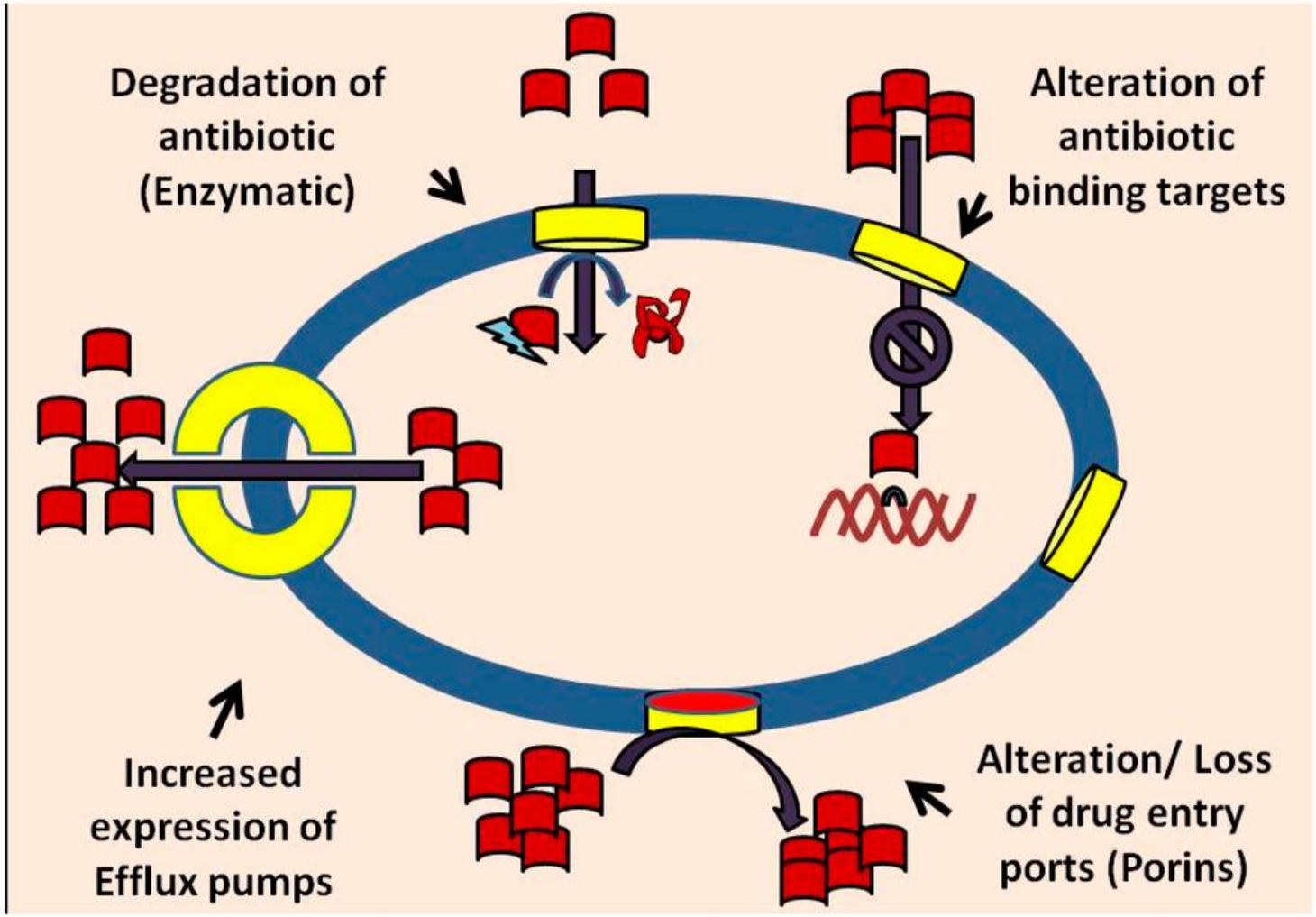
Testing Conditions

Medium:	Disk diffusion: MHA
Broth dilution:	CAMHB
Agar dilution:	MHA
Inoculum:	Growth method or direct colony suspension, equivalent toa 0.5 McFarland standard
Incubation:	35 ± 2 °C; ambient air;
Disk diffusion:	16 to 18 hours
Dilution methods:	16 to 20 hours

Minimal QC Recommendations (See Tables 3A and 4A for acceptable QC ranges.)
Escherichia coli **ATCC® 25922**
Pseudomonas aeruginosa **ATCC® 27853**
Escherichia coli **ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)**

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Breakpoints, nearest whole mm			MIC Interpretive Standard (µg/mL)		
			S	I	R	S	I	R
PENICILLINS								
(5) *Rx:* The susceptible category for these drugs implies the need for high-dose therapy for serious infections caused by *P. aerugi* associated with clinical failure.								
A	Piperacillin	100 µg	≥ 18	–	≤ 17	≤ 64	–	≥ 128
B	Ticarcillin	75 µg	≥ 15	–	≤ 14	≤ 64	–	≥ 128
O	Azlocillin	75 µg	≥ 18	–	≤ 17	≤ 64	–	≥ 128
O	Carbenicillin	100 µg	≥ 17	14–16	≤ 13	≤ 128	256	≥ 512
O	Mezlocillin	75 µg	≥ 16	–	≤ 15	≤ 64	–	≥ 128
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS								
See comment (4).								
B	Piperacillin-tazobactam	100/10 µg	≥ 18	–	≤ 17	≤ 64/4	–	≥ 128/4
O	Ticarcillin-clavulanic acid	75/10 µg	≥ 15	–	≤ 14	≤ 64/2	–	≥ 128/2
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
A	Ceftazidime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32
B	Cefepime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32
MONOBACTAMS								
B	Aztreonam	30 µg	≥ 22	16–21	≤ 15	≤ 8	16	≥ 32
CARBAPENEMS								
B	Imipenem	10 µg	≥ 16	14–15	≤ 13	≤ 4	8	≥ 16
B	Meropenem	10 µg	≥ 16	14–15	≤ 13	≤ 4	8	≥ 16
LIPOPEPTIDES								
O	Colistin	10 µg	≥ 11	–	≤ 10	≤ 2	4	≥ 8
O	Polymyxin B	300 units	≥ 12	–	≤ 11	≤ 2	4	≥ 8
AMINOGLYCOSIDES								
A	Gentamicin	10 µg	≥ 15	13–14	≤ 12	≤ 4	8	≥ 16
A	Tobramycin	10 µg	≥ 15	13–14	≤ 12	≤ 4	8	≥ 16
B	Amikacin	30 µg	≥ 17	15–16	≤ 14	≤ 16	32	≥ 64
O	Netilmicin	30 µg	≥ 15	13–14	≤ 12	≤ 8	16	≥ 32
FLUOROQUINOLONES								
B	Ciprofloxacin	5 µg	≥ 21	16–20	≤ 15	≤ 1	2	≥ 4
B	Levofloxacin	5 µg	≥ 17	14–16	≤ 13	≤ 2	4	≥ 8
U	Lomefloxacin or	10 µg	≥ 22	19–21	≤ 18	≤ 2	4	≥ 8
U	ofloxacin	5 µg	≥ 16	13–15	≤ 12	≤ 2	4	≥ 8
U	Norfloxacin	10 µg	≥ 17	13–16	≤ 12	≤ 4	8	≥ 16
O	Gatifloxacin	5 µg	≥ 18	15–17	≤ 14	≤ 2	4	≥ 8

Bacterial Antibiotic Resistance Mechanisms



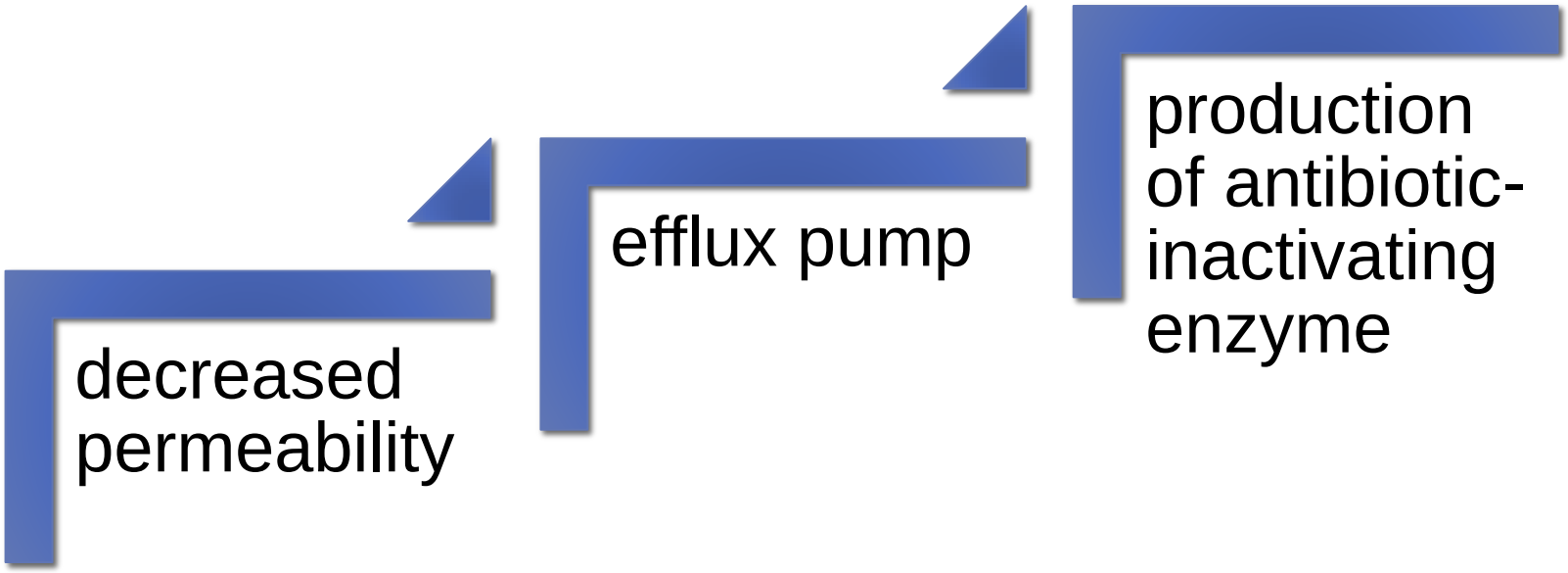
Antibiotic Resistance Mechanisms of *P. aeruginosa*

Intrinsic & Acquired

Intrinsic- A consequence of a large selection of genetically encoded mechanisms,

Acquired- Resistance that is achieved via the acquisition of additional mechanisms or is a consequence of mutational events under selective pressure.

Intrinsic resistance of *P. aeruginosa*



Acquired resistance of *P. aeruginosa*

Enzyme Production

Detection of Resistance Genes

All resistant *P. aeruginosa* isolates were screened for the presence of

* CMY,
* AAC(6′)-Ib,
* armA, rmtB, rmtC, rmtD,
* IMP & VIM

by PCR

Oligonucleotide Primers Used in This Study

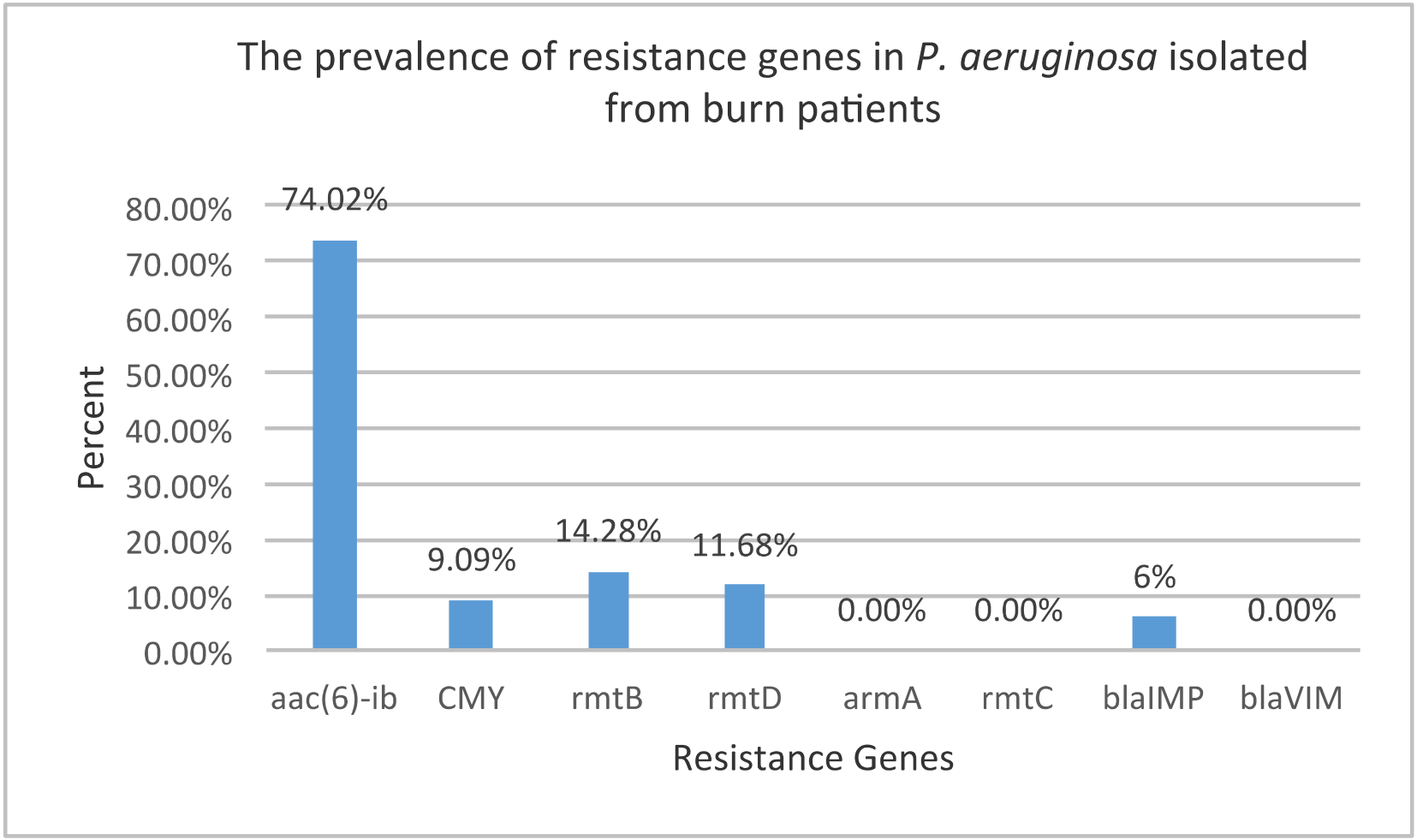
Nucleotide Sequence	Primer	Target	PCR Conditions			Size (bp)
			Denaturing	Anneal	Extension	
(5´-ATGATGAAAAAATCGTTATG-3´) (5´-TTGTAGCTTTTCAAGAATGC-3´)	CMY-F, CMY-R	CMY	94°C, 45 s	55°C, 45 s	72°C,45 s	635
(5´-GCTTTCTGCGGGCGATGTAA-3´) (5´-ATGCAATGCCGCGCTCGTAT-3´)	rmtB-F, rmtB-R	rmtB	94°C, 45 s	55°C, 45 s	72°C, 45 s	173
(5´-CGAAGAAGTAACAGCCAAAG-3´) (5´-ATCCCAACATCTCTCCCACT-3´)	RmtC-F, RmtC-R	rmtC	94°C, 45 s	55°C, 45 s	72°C, 45 s	711
(5´-ATTCTGCCTATCCTAATTGG-3´) (5´-ACCTATACTTTATCGTCGTC-3´)	ArmA-F, ArmA-R	armA	94°C, 45 s	55°C, 45 s	72°C, 45 s	315
(5´-CGGCACGCGATTGGGAAGC-3´) (5´-CGGAAACGATGCGACGAT-3´)	RmtD-F, RmtD-R	rmtD	94°C, 30 s	55°C, 30 s	72°C, 30 s	401
(5´-TTGCGATGCTCTATGAGTGGCTA-3´) (5´-CTCGAATGCCTGGCGTGTTT- 3´)	aac(6´)-Ib-F, aac(6´)-Ib-R	aac(6´)-Ib	94°C, 45 s	55°C, 45 s	72°C, 45 s	482

Nucleotide Sequence Accession Number

The nucleotide sequence data reported in this paper have been submitted to the Gene Bank sequence database and assigned accession No.

JX648311 & JX644173

RESULTS


The prevalence of resistance genes in P. aeruginosa isolated from burn patients
Percent
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
74.02%
9.09%
14.28%
11.68%
0.00%
0.00%
6%
0.00%
aac(6)-ib
CMY
rmtB
rmtD
armA
rmtC
blaIMP
blaVIM
Resistance Genes

Results…

- **All *P. aeruginosa* isolates were resistant to**
  - Pen + B Lactamase inhib: Carbenicillin, & Piperacillin/Tazobactam
  - Ceph: Ceftriaxone, Cefepime,
  - Carb: Meropenem,
  - Monob: Aztreonam,
  - AGs: Amikacin, Tobramycin,
  - FQs: Ciprofloxacin,
- 77% resistant to Imipenem & Ceftazidime
- 49% resistant to Gentamicin

This study detected MDR in all P. aeruginosa isolates, including resistance to β-lactams, AGs, FQs and CBPs.

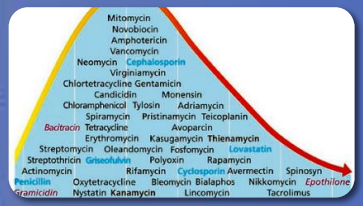
WHAT CAN WE DO TO COMBAT THIS GROWING THREAT?

Four Core Actions to Fight Resistance

PREVENTING INFECTIONS, PREVENTING THE SPREAD OF RESISTANCY

TRACKING RESISTANCE PATTERNS.

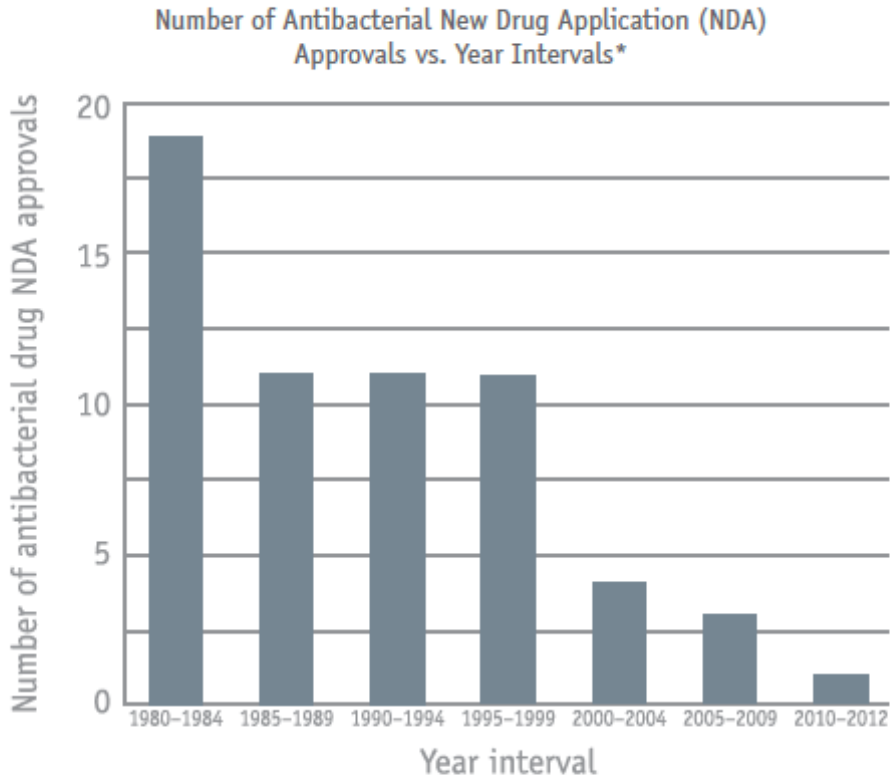
IMPROVING USE OF ANTIBIOTICS.



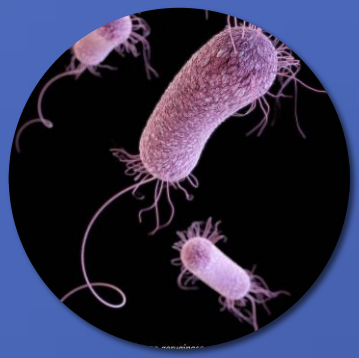
DEVELOPING NEW ANTIBIOTICS AND DIAGNOSTIC TESTS

Tomorrow's Antibiotics: The Drug Pipeline

The number of new antibiotics developed and approved has steadily decreased in the past three decades, leaving fewer options to treat resistant bacteria.



*Intervals from 1980–2009 are 5-year intervals; 2010–2012 is a 3-year interval. Drugs are limited to systemic agents.
Data courtesy of FDA's Center for Drug Evaluation and Research (CDER).



Bacteria will inevitably find ways of resisting the antibiotics we develop, which is why aggressive action is needed now to keep new resistance from developing and to prevent the resistance that already exists from spreading.

MISUSE OF ANTIBIOTICS
IS
THE SINGLE MOST IMPORTANT FACTOR LEADING TO ANTIBIOTIC RESISTANCE AROUND THE WORLD


IRAN

"THANK YOU FOR YOUR ATTENTION"