Precision Medicine Series:
Genomic of Bacterial Resistance in Clinical Practice

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Veritas, Probitas, Justitia

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IMERI
INDONESIAN MEDICAL EDUCATION AND RESEARCH INSTITUTE

The 16th PIPKRA, 8 February, 2019

Presentation Outline

- What is & Why Industry 4.0?
- Challenges in infectious lung diseases
- Precision medicine in infectious lung diseases
- **Bacterial genomics as backbone**
- Workflow & target outputs
- Prospects & controversies in Indonesia
- On-going project & its collaborators
- Take home message

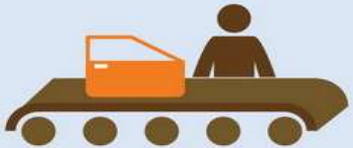
Kementerian Perindustrian
REPUBLIK INDONESIA

INDUSTRI 4.0 DAN SEKTOR PRIORITAS

Indonesia bersiap menghadapi era Revolusi Industri ke-4 atau Industri 4.0, dalam upaya meningkatkan daya saing dan produktivitas industri manufaktur nasional

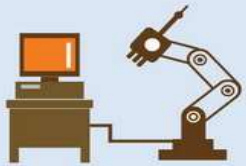
Industri 1.0 (mulai 1784)

Penggunaan mesin uap dalam industri



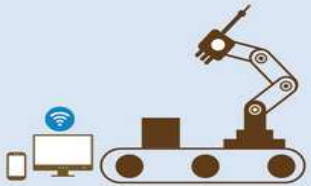
Industri 2.0 (mulai 1870)

Penggunaan mesin produksi massal tenaga listrik/BBM



Industri 3.0 (mulai 1969)

Penggunaan teknologi informasi dan mesin otomasi



Industri 4.0 (diperkenalkan 2011)

Mesin terintegrasi jaringan internet (internet of things)

5 Sektor Industri Prioritas menuju Industri 4.0

9,23%
6,14%

Industri Makanan dan Minuman

4,53%
1,74%

Industri Kimia

3,76%
1,11%

Industri Tekstil dan Pakaian Jadi

3,68%
1,82%

Industri Otomotif

2,79%
1,86%

Industri Elektronika

Kelima sektor industri tersebut memberi kontribusi **12,67%** terhadap total PDB atau **70,86%** terhadap PDB industri pengolahan nonmigas tahun 2017.

Keterangan : Pertumbuhan 2017 Kontribusi terhadap PDB

Challenges in Infectious Lung Diseases

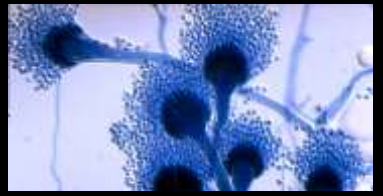
Nontuberculous Mycobacteria

MDRO agents

Viral lung diseases

All forms of TB

1. Difficult to **prevent**
2. Slow to **diagnose**
3. Difficult to **treat**
4. Poor **prognosis/sequelae**



Fungal/parasitic lung diseases

Eur Resp J. 2017;49:1602086
Respiration. 2016;92:199-214

Precision Medicine in Infectious Lung Diseases

- **Precision medicine** → understanding a person's bio-physio-socio-genomic status (BPSG) → to prevent & treat disease → improve population health

- BPSG → Big data of patient & microbiological characteristics

- Impacts of Big Data in infectious lung diseases:
  - Microbiological rapid diagnostic test (RDT), biomarkers
    → prevention, categorizing risks, therapy, monitoring, prognosis

Am J Respir Crit Care Med. 2018;198(12):e116-36
Clin Microbiol Infect. 2018;24(12):1264-72
Trends Microbiol. 2017;25(11):875-6

BPSG in Precision Medicine

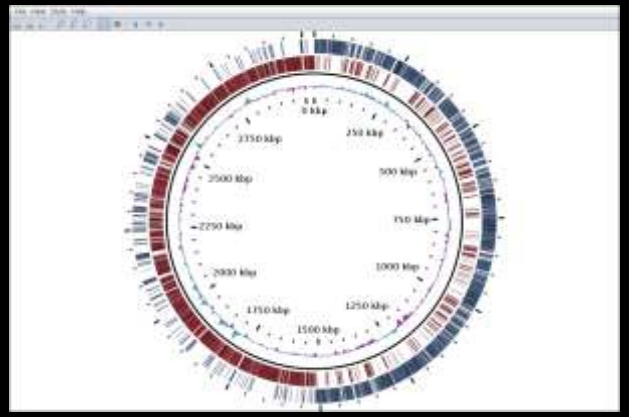
Clinical data	Supportive data	Biomolec data
● Chief complaint ● History of illness ● Family history ● ***Socio-religio-psycho-economic stat*** ● Vital signs ● ***Anthropometric*** ● Etc	● Routine blood ex ● Blood gas analys ● Spirometric ● Radiologic ● Microbiologic ● Cytologic ● Histopathologic ● Etc	● DNA ● RNA ● Amino ac ● Epigenetic ● Immunologic ● Elements/subst ● Etc
C	**S**	**B**

BMC Med Inform Decis Mak. 2018;18(1):139
BMC Bioinformatics. 2018;19(Suppl 10): 257-67
Prod Oper Manag. 2018;27:1647-64

C12s S13g B86g | C1a S1c B2c | C1h S2v B2b

C34sf S14j B84g | C11a S19c B24c | C31h S26v B22b

BPSG + Pathogenic Genomic Features



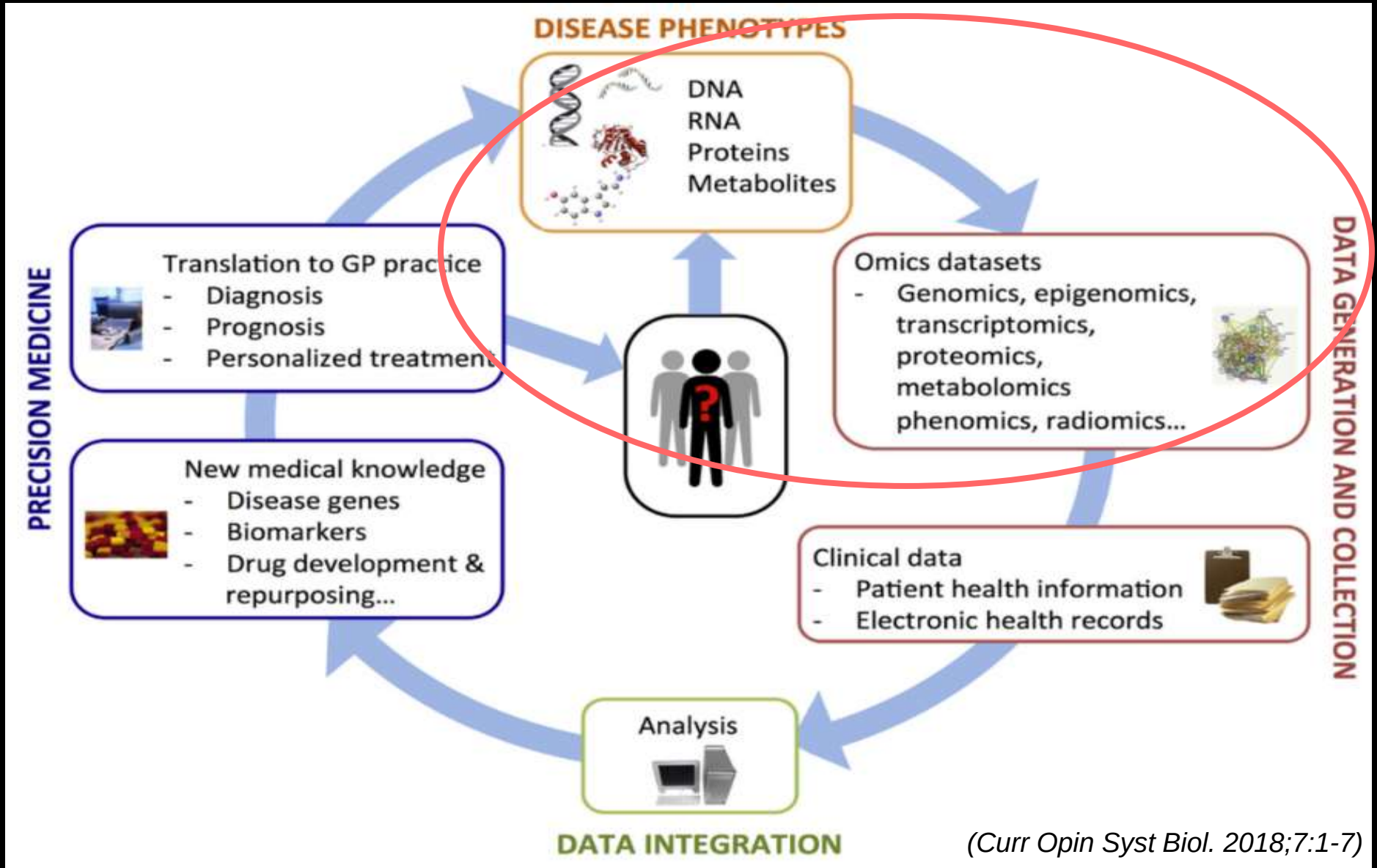
Pathogenic Resistome

Pathogenic Virulome

C **S** **B** **P**

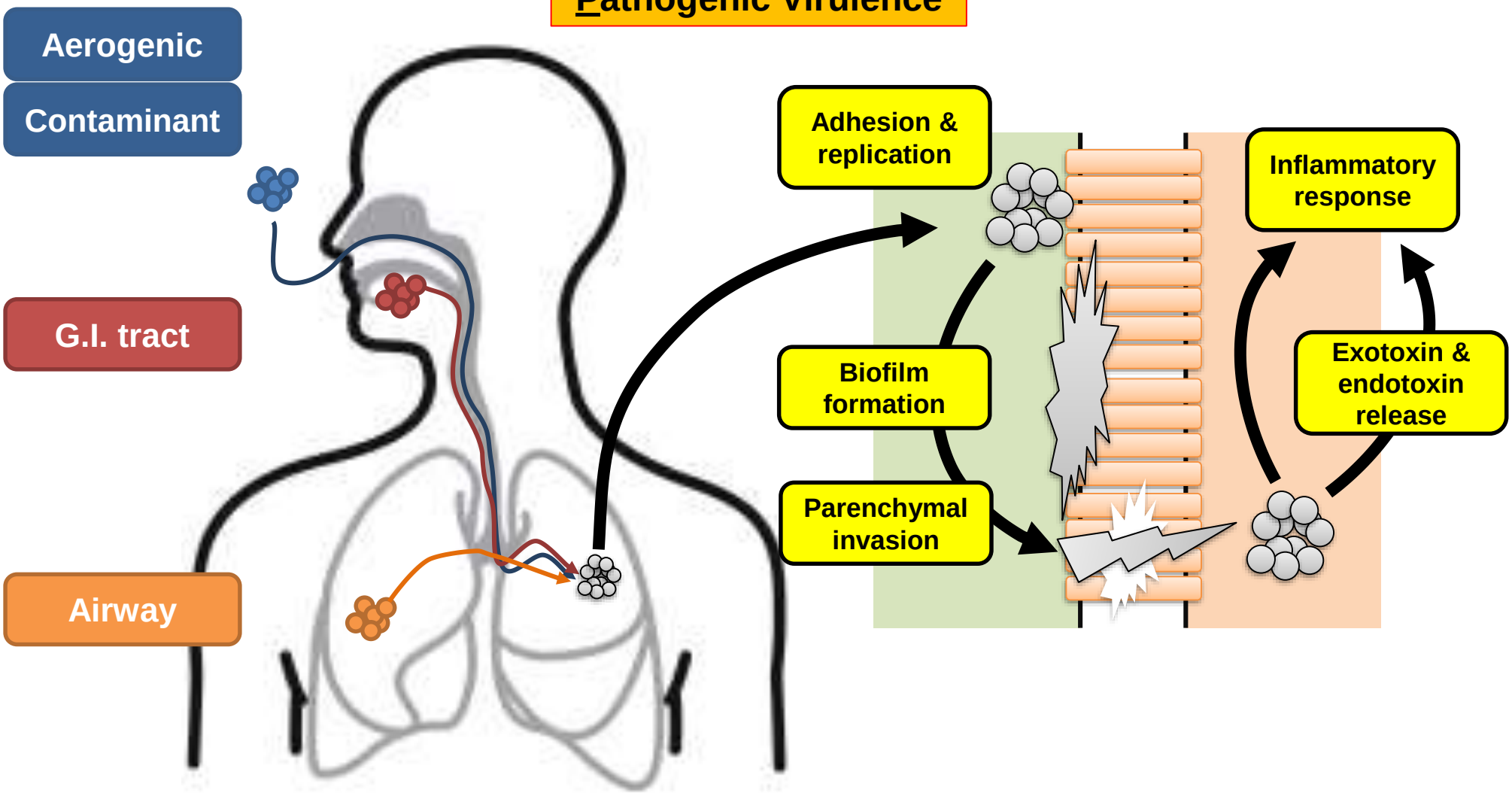
Precision Medicine

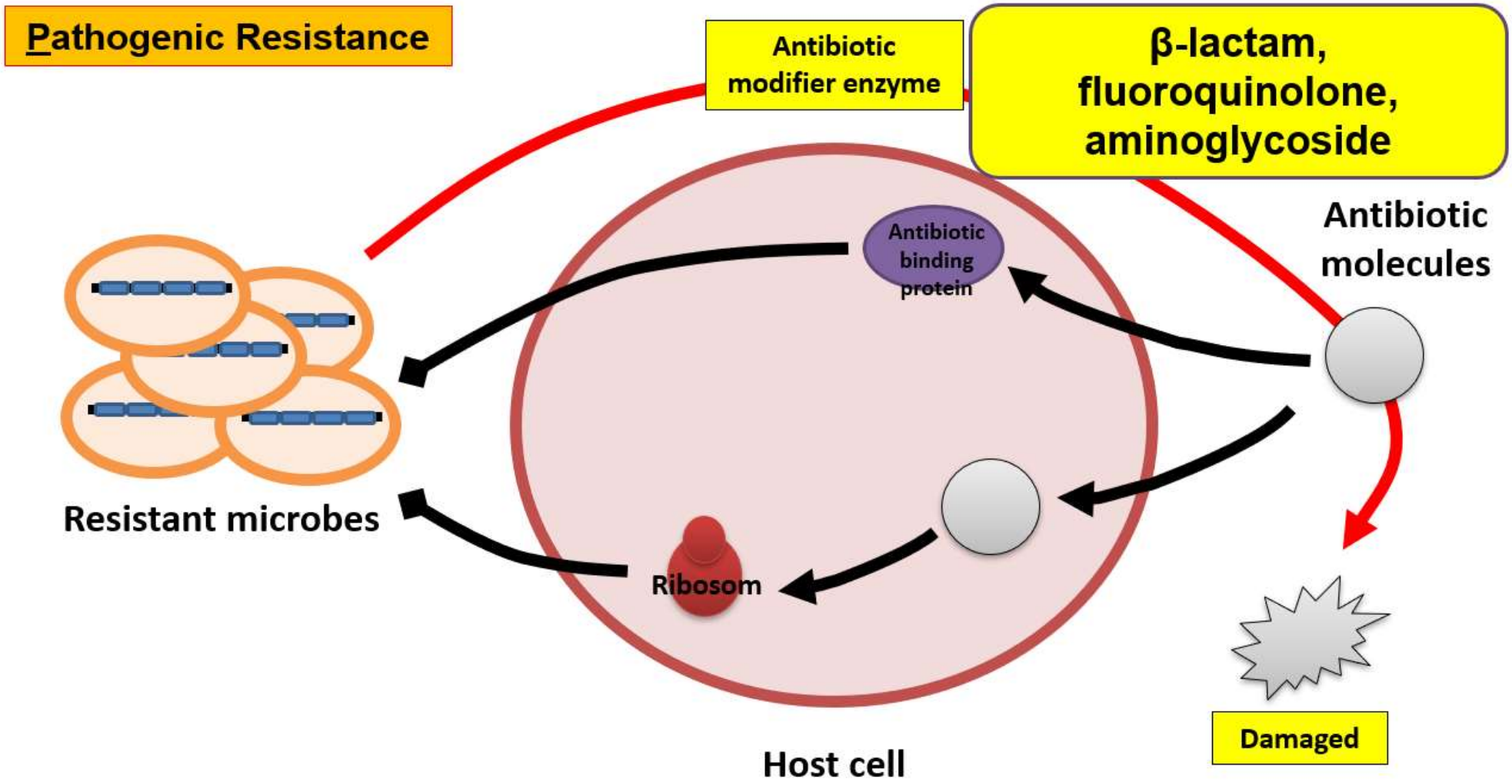
Trends Microbiol. 2017;25(11):875-6



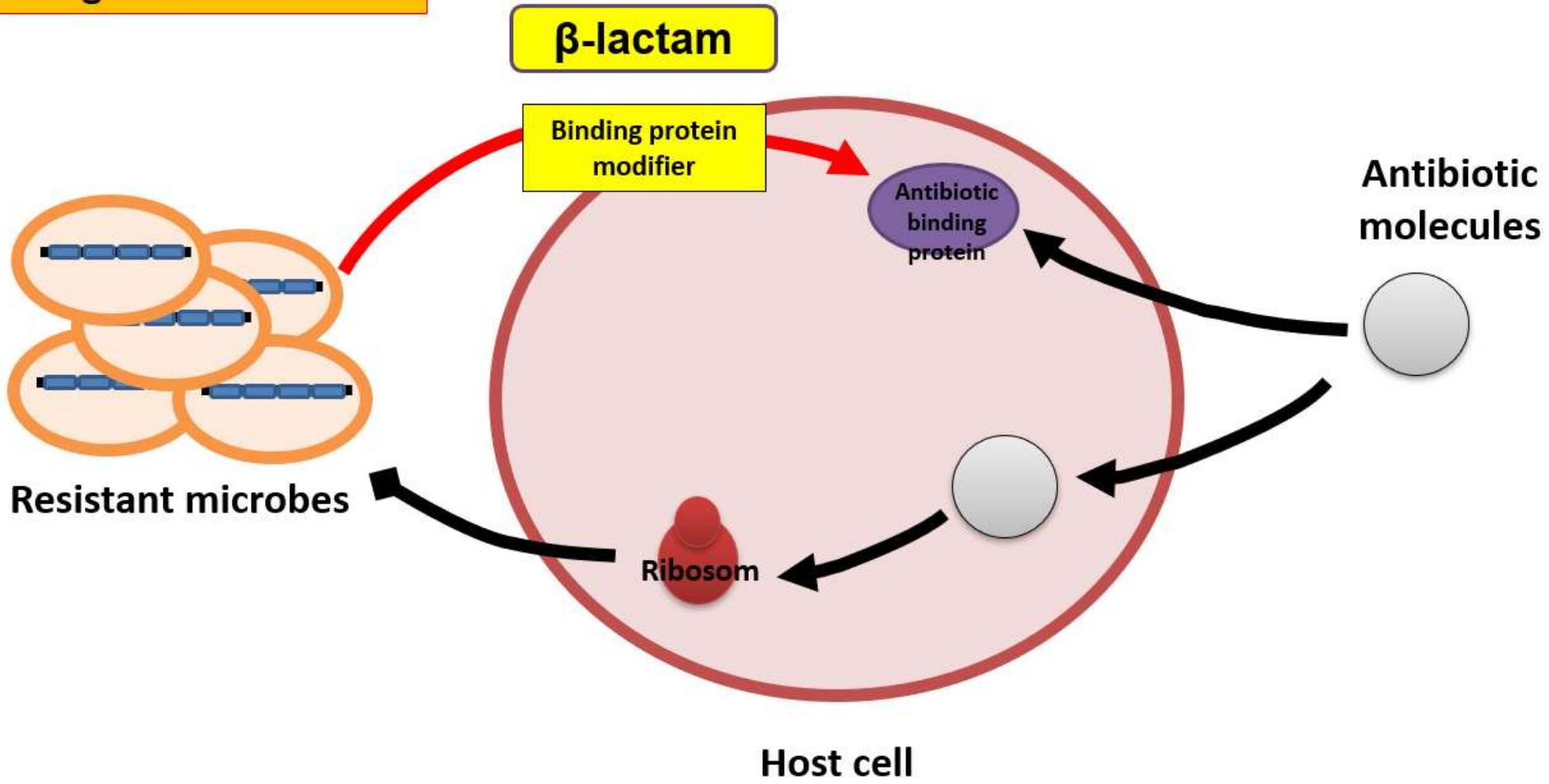
(Curr Opin Syst Biol. 2018;7:1-7)

Pathogenic Virulence

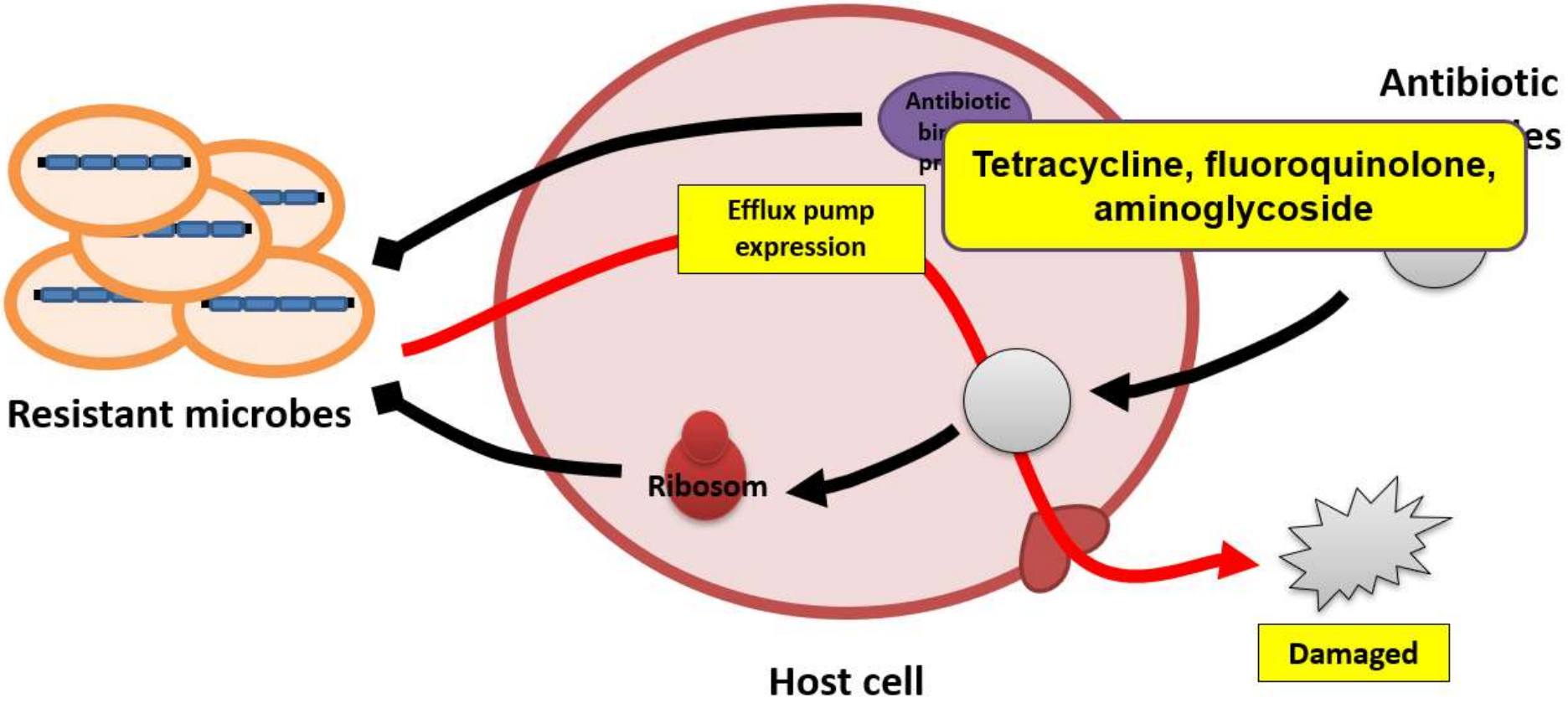



Pathogenic Resistance
Antibiotic modifier enzyme
β-lactam, fluoroquinolone, aminoglycoside
Antibiotic molecules
Antibiotic binding protein
Resistant microbes
Ribosom
Host cell
Damaged

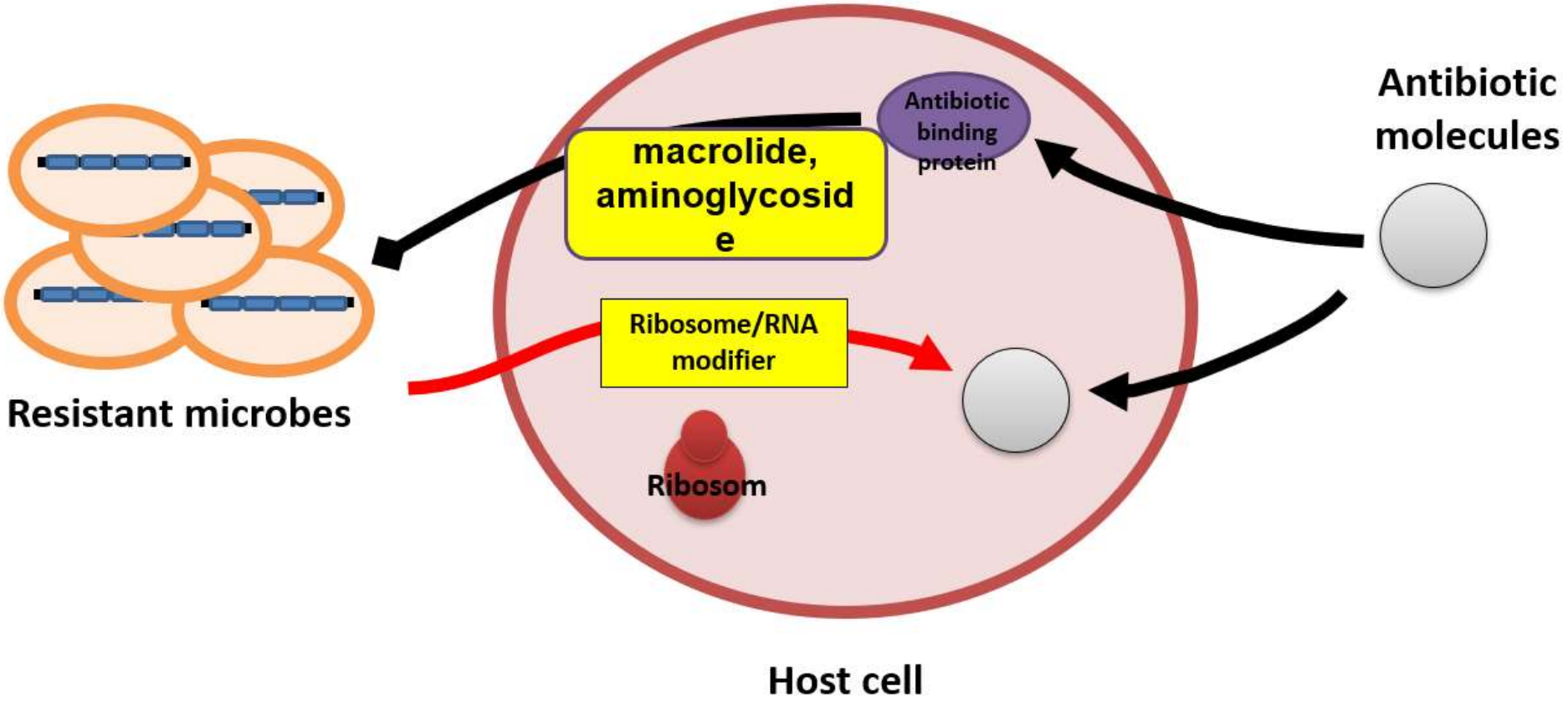
Pathogenic Resistance



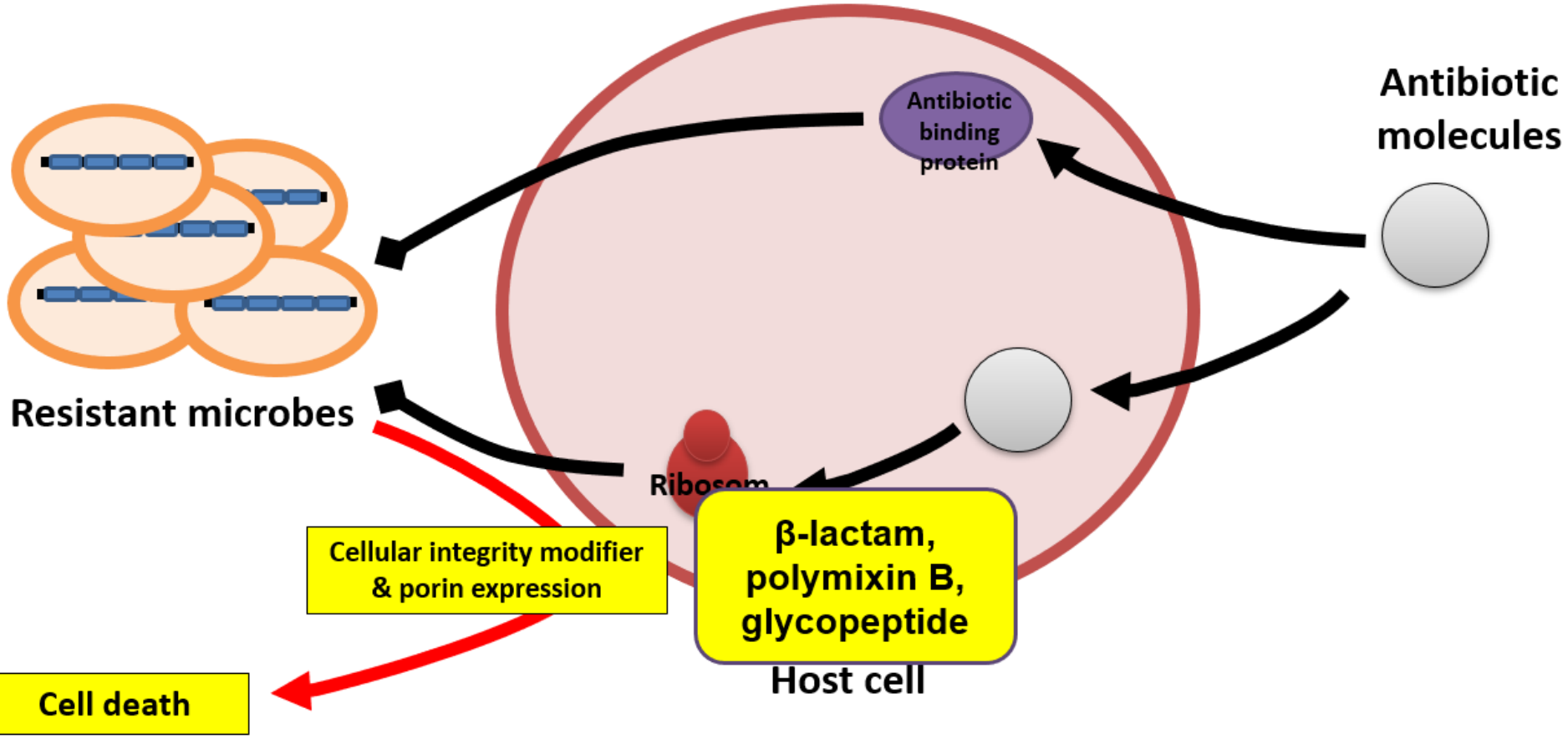
Pathogenic Resistance



Pathogenic Resistance



Pathogenic Resistance

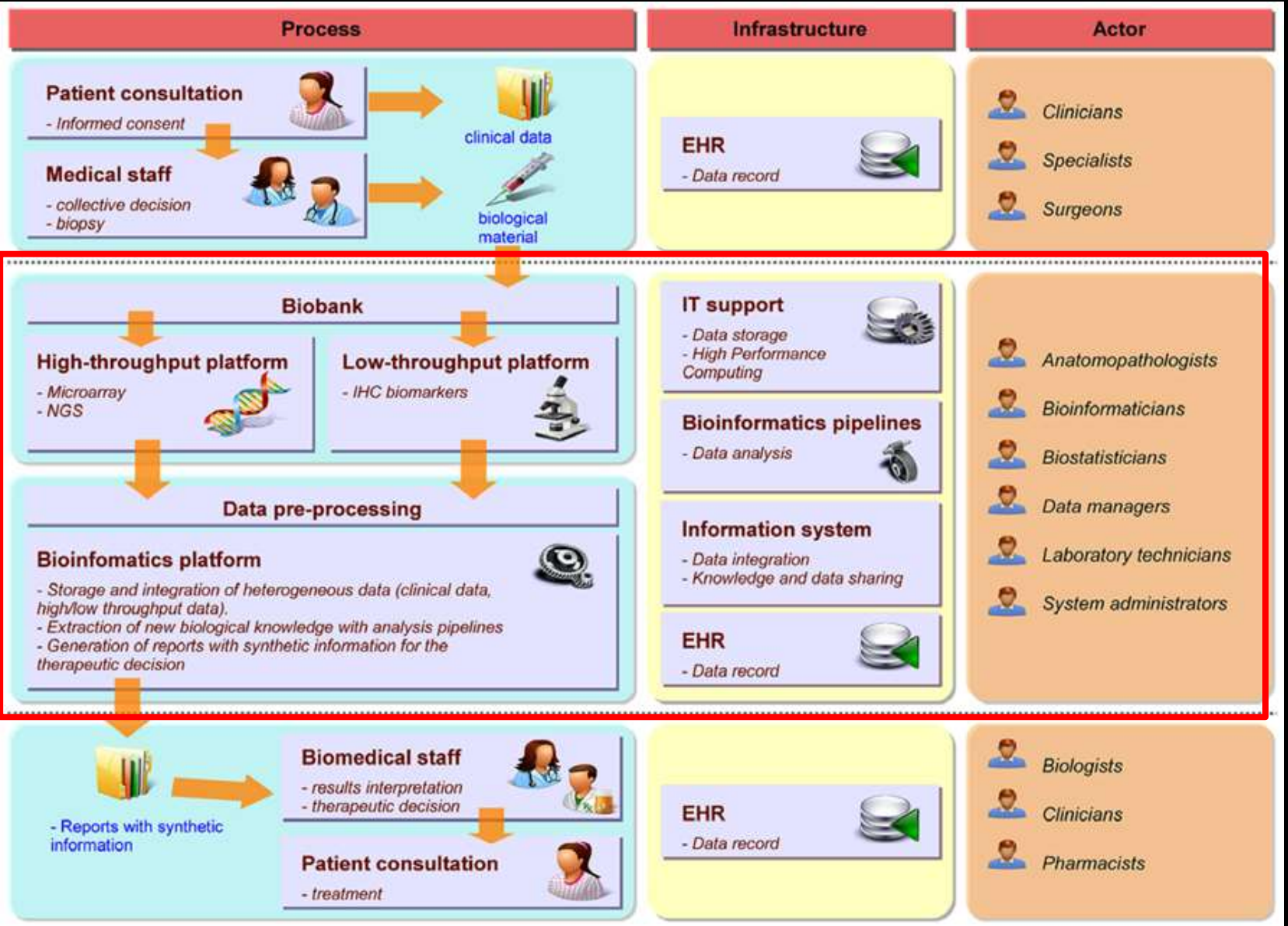


Antibiotic		ESKAPE*						
		ABB	EBS	ECS	KLP	PMA	SCA	
β-lactam	Penicillin						+	
	Ampicillin			+				
	Cefoxitine						+	
	Cefotaxime	+						
	Ceftazidime					+		
	Cefoperazon sulbaktam				+	+		
	Cefepime					+		
	Piperacilin	+			+	+		
	Imipenem	+			+	+		
	Meropenem	+			+			
Glycopeptide	Vancomysin			+				
Aminoglycoside	Gentamicin	+	+	+	+	+	+	
	Amikacin	+	+		+	+		
Macrolide	Erythromycin						+	
Folic inhibitor	Cotrimoxazole	+			+			
Fluoroquinolone	Ciprofloxacin		+		+	+		

**ABB: A. baumannii, EBS: Enterobacter spp, ECS: Enterococcus spp, KLP: K. pneumoniae, PMA: P. aeruginosa, SCA: S. aureus.*

Pathogenesis	Responsible Genes*
Virulence Factors	
Adhesions	*ABB: gacSA; EBS: fim, irp2; ECS: ace, asa, efaA; KLP: cps, ipr2; SCA: clf, fnbp, spa*
Colony formation	*ABB: abaR; EBS: irp2; ECS: ebp; KLP: fur, ipr2*
Biofilm formation	*ABB: atfA, abaR, adeRS, bfmRS; ECS: ebp, esp, gelE, hyl; KLP: celB, lux, oxyR*
Parenchymal invasion	*ABB: pld; ECS: ace*
Exotoxin	*ABB: exoSTUY, toxAS, lasAB, aprA, plhHN; EBS: cdiA; ECS: cyl; SCA: hla, hlb, lukS, lukF, se, tst*
Endotoxin	*ABB: mazEF; EBS: stx1*
Resistance Factors	
Antibiotic binding protein modifier	*EBS: bla; ABB: gyr; KLP: bla; PMA: gyr; SCA: bla, mecA*
Antibiotic modifier enzyme production	*EBS: aac, aad, aph; KLP: ampC; PMA: ampC; SCA: tetX*
Porin expression	*ABB: carO, ompA*
Efflux pump expression	*ECS: erm; PMA: muxABC; SCA: norABC, tet38, tetK, tetM;*
Ribosome & RNA modifier	*EBS: armA, rmtBCF; ECS: erm*
Cellular integrity modifier	*ABB:lpxADC, pmrABC; ECS: vanAB; PMA:psrA*

Clin Microb Rev. 2013;26(2):185-230; Microbial pathogens and strategies for combating them: science, technology and education. Vol. 1. Badajoz: Formatex Research Center; 2013.p.702-10; Indian J Pathol Microbiol. 2013;56(3):238-42; Structure. 2014;22(5):707-18; Mem Inst Oswaldo Cruz. 2014;109(4):502-5; JCM. 2014;52(7):2346-51; Journal of Intensive Care. 2014;2(1):10; Pathology. 2015;47(3):276-84; PLoS ONE. 2015;10(9):e0138360; Microb Pathog. 2016;96(SC):15-9; Braz J Infect Dis. 2016;20:127-33; Microbiol Spectr. 2016;4(1); Al-Qadisiyah Journal of Pure



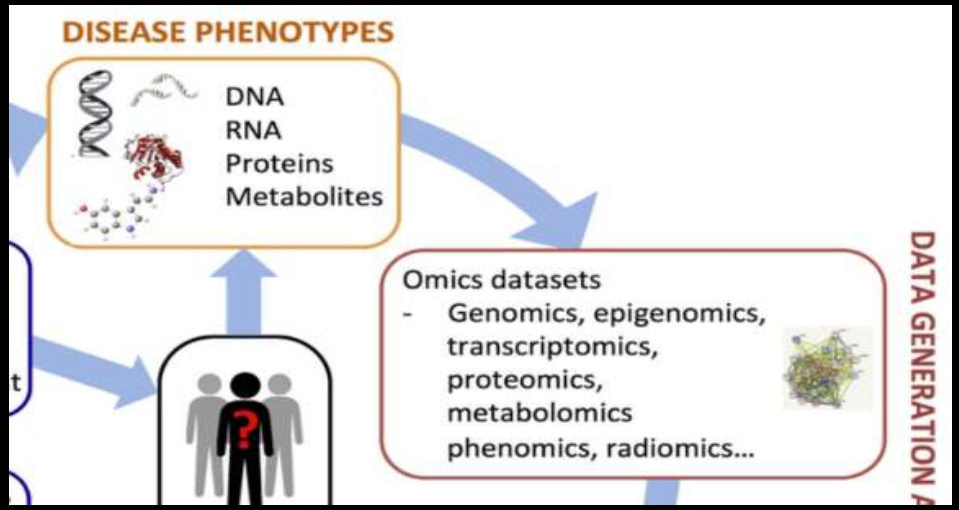
(Front Genet. 2014;5:152)

Typical Unique Inputs in Infectious Lung Studies

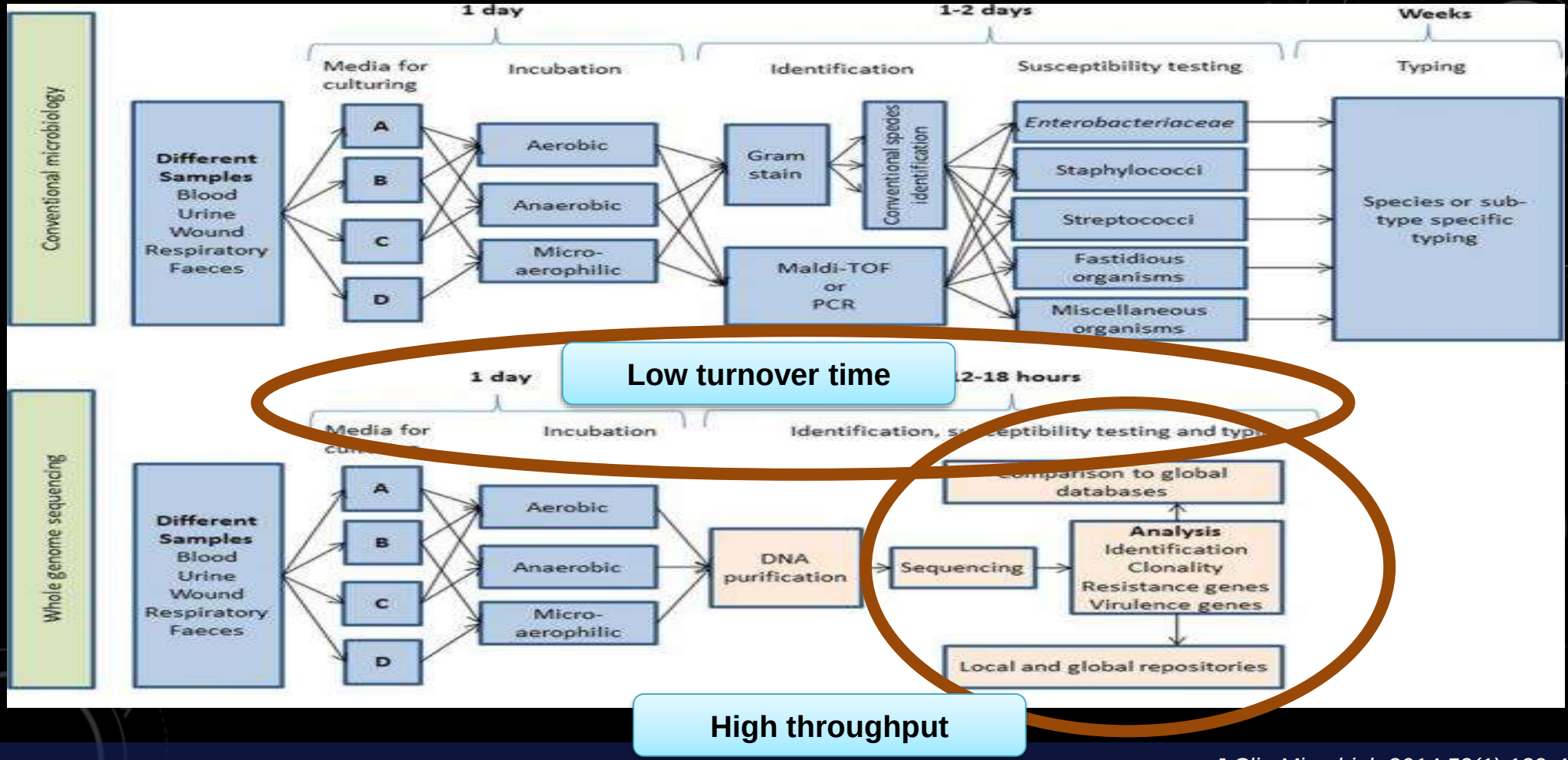
- Non-respiratory (blood, urine, sweat, hair)
- Respiratory (sputum, swab, lavage, biopsy, exhaled breath condensate, pleural specimens)

Proc Natl Acad Sci U S A. 2018;115(52):E12353-62
Appl Environ Microbiol. 2017;83(12):e00515-17

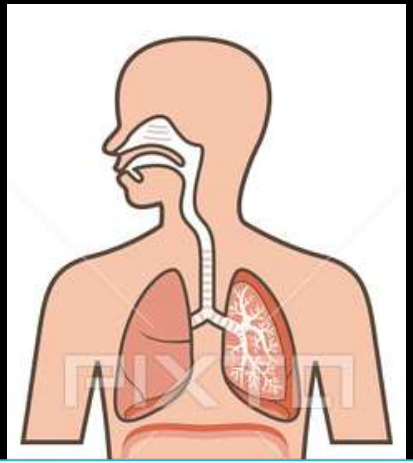
Typical Instruments

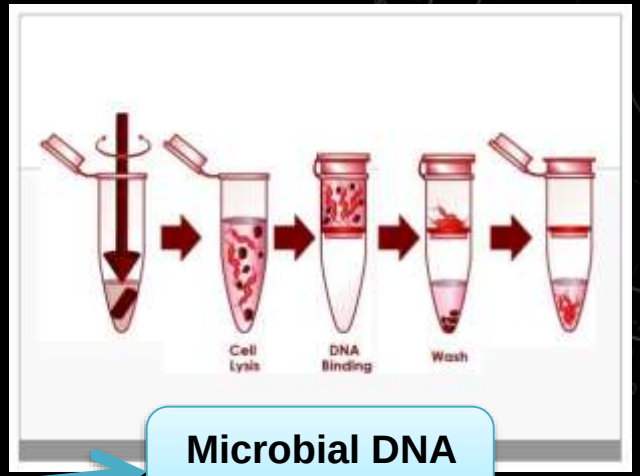


Typical workflow in genomic bacterial lung diseases



J Clin Microbiol. 2014;52(1):139-46





Airway secretes (sputum, swab, aspirate)

Appropriate culture medium

Microbial DNA extraction

Library preparation (incl. amplification, condensation, label integration)

Sequencing

Microb Inform Exp. 2013;3(1):2.
Int J Mol Sci. 2017;18(9).
Clin Infect Dis. 2012;54 Suppl 2:S153-8.
J Clin Microbiol. 2014;52(1):139-46.

Sequencer

Sequence (raw) reads

De novo assembly

Computational biology

Reference mapping

Previously known sequence (required)

Contigs/scaffold/ genome

Microbiome epidemiological data

Manual comparison, phylogenic analysis tools, *in silico* genotyping

Signature comparison analysis

Annotated genome

Virulence & resistance signatures

Annotation

Draft genome

Microb Inform Exp. 2013;3(1):2.
Int J Mol Sci. 2017;18(9).
Clin Infect Dis. 2012;54 Suppl 2:S153-8.
J Clin Microbiol. 2014;52(1):139-46.

- **Clinical Implications: transmissions**

Researchers (year)	Country/Region	NGS Platforms	Microbiome	Results
Brodrick (2016)		Illumina HiSeq	VRE	Two VRE clones were identified and both were responsible in disease dissemination in a healthcare unit
Azarian (2015)		Illumina HiSeq	MRSA	Two MRSA clones were identified and both were responsible in disease dissemination in neonatal intensive care
Pratomo (2014)		Illumina MiSeq	MRSA	More than two MRSA clones were identified. Disease dissemination between high care & non-high care units was identified.
Snitkin (2012)		Roche 454	KLP	Several KLP clones were responsible in disease dissemination
Dettman (2013)	 	Illumina GAII	PMA	Intercontinental transmissions were identified

Genome Med. 2016;8(1):4; Infect Control Hosp Epidemiol. 2015;36(7):777-85; Respirology. 2014;19(Supp.3):91; Sci Transl Med. 2012;4(148):148ra16; Proc Natl Acad Sci U S A. 2013;110(52):21065-70.

- **Clinical impacts: biomarker/virulence signature discovery**

Researchers (year)	Country/Region	NGS Platforms	Microbiome	Results
Pratomo (2017)		Illumina MiSeq	MRSA	Two virulence signatures were significantly correlated to the mortality of ICU patients
Pratomo (2017)		Illumina MiSeq	MRSA	MICs of aminoglycoside, macrolide & tetracycline were correlated with positive detection of genomic virulence signatures
Kos (2015)		Illumina HiSeq & MiSeq	PMA	Correlations between positive detection of genomic virulence signatures & meropenem – levofloxacin resistance were identified
Arena (2014)		Ion Torrent PGM	KLP	Disease transmissions were inferred from identifying genomic virulence signature variants

Respirology. 2017;22(S3):174; Hiroshima J Med Sci. 2017;66; Antimicrob Agents Chemother. 2015;59(1):427-36. J Clin Microbiol. 2014;52(3):987-90.

Drawbacks:

- Highly technical → **collaborative attitude** inevitable
- **Initial** preparation & infrastructure → high cost
- Vast types of NGS platform → **standards → limited to none**

Current quick fix:

- **NGS best practice standards** → i.e. GAGE-B & GABenchToB → still in limited use
- **Targeted** genomic signature analysis, instead of whole-genome analysis

PLOS ONE. 2016;11(5):e0155770.
Clin Microbiol Infect. 2017;23(1):2-22.
PLoS One. 2014;9(9):e107014.
Bioinformatics. 2013;29(14):1718-25

SEQUENCING METHODOLOGY

Comparable Variables	1st Gen Sequencing	NGS
Parallel sequencing	No, 1 unidirectional sequence per run	Yes, 1 mil – 80 bil bidirectional sequences per run
Output sequence (base pairs/bps)	400 – 900	50 – 700
Accuracy	99.9%	98.0 – 99.9%, from deep sequencing
Reagent & equipment cost	$$	$$$$
Cost per run	$$$$	$
Turnover time	20 mins – 30 hrs	2 hrs – 11 days
Confounding factor	Relatively none	Tandem repeat sequence

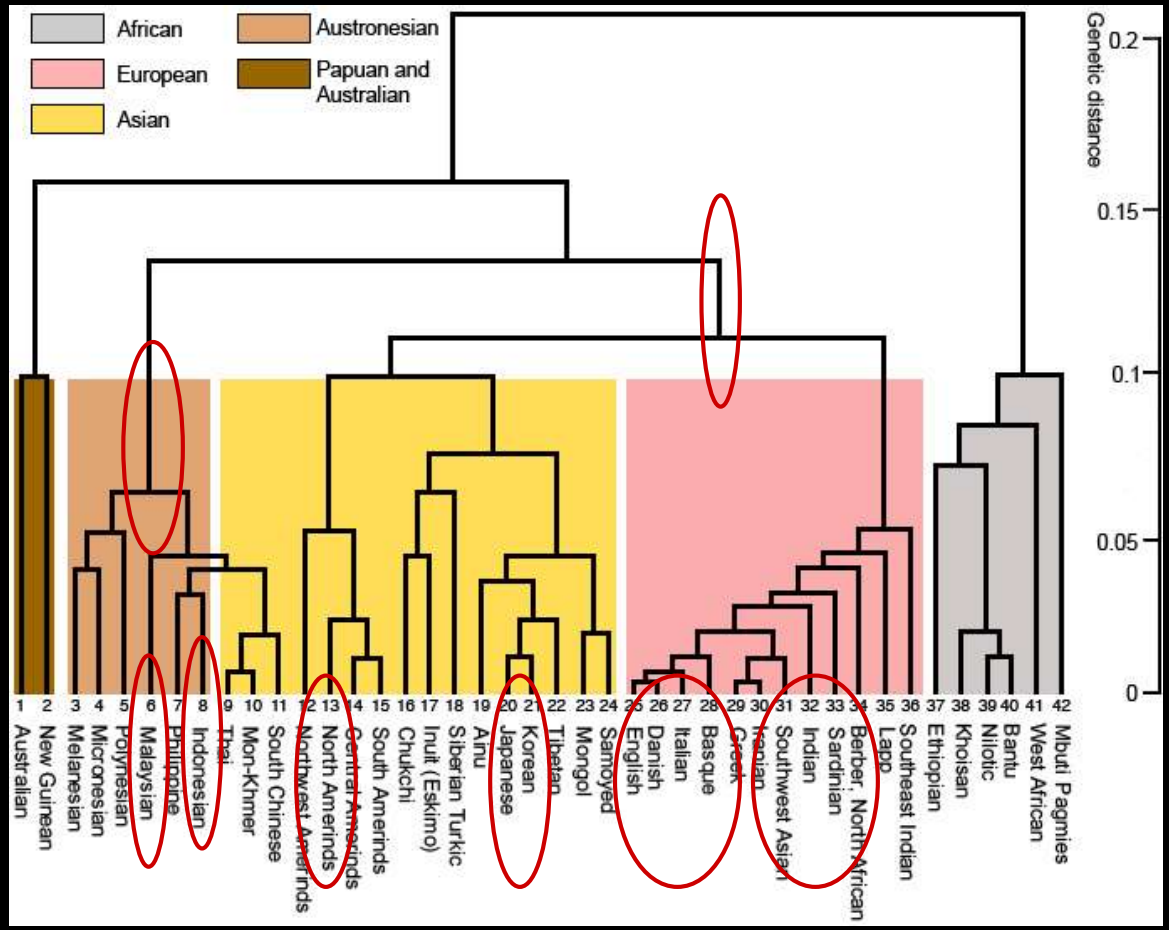
Global/Regional Databases in Lung Studies

- Czech Multicentre Research Database of COPD
  http://chopn.registry.cz/index-en.php
- ICGC Cancer Genome Projects http://icgc.org/
- Allergy and Asthma Portal http://allergen.innatedb.com/
- BGI Sentis https://www.bgi.com
- Columbia Combined Cancer Panel (CCCP)
  https://www.pathology.columbia.edu/diagnostic-specialties/laboratory-medicine-division/personalized-genomic-medicine/pgm-oncology-3
- General research portals: NCBI, DDBJ, etc

Local Databases in Lung Studies

- SRiKandI (Dharmais Hospital Jakarta) → limited to no access
- Persahabatan Hospital Jakarta → limited to no access
- Balitbangkes → limited to no access
- Jogja Cancer Registry (UGM) http://canreg.fk.ugm.ac.id/home/

Prospect in Indonesia



The History and Geography of Human Genes, 1994

Better understanding:

- Disease
- Risk factors
- Inheritance
- Culture

New discovery:

- Mutation, gene, protein, epigenetics, etc
- Biomolec pathway
- Drugs

PATIENT-ORIENTED APPROACH

DeNA GAMES

- HOME
- NEWS
- TRAILERS
- GAMES

Final Fantasy Record Keeper

Download on the App Store

GET IT ON Google Play

Official Website

NEWS

MORE

http://dena.com/jp/

JAPAN REAL TIME

Games Provider DeNA Moves Into DNA Testing

By Yoko Sudo

Jun 5, 2014 11:49 am JST

0 COMMENTS

http://on.wsj.com/SvJsA9
http://dena.com/jp/

MYCODE マイコード

1.入力 | 2.確認 | 3.完了

注文情報の入力

商品選択

ヘルスケア **29,800円**(税別)

数量 1

病気と体質に関する全検査（280項目）が入ったセット

病気（3大疾病のがん・心筋梗塞・脳梗塞 等）と体質（長生き・肥満・肌質等）の遺伝的傾向を知るフルパッケージ

「がんパック」の内容を全て含んでいます

【セット割引対象商品】詳しく見る›

ヘルスケア(検査レポートブック付)

33,800円(税別)

数量 0

病気と体質の全検査(ヘルスケア)に主要項目の冊子付属の特別セット

病気（がん・心筋梗塞 等）と体質（長生き・肥満 等）の遺伝的傾向が分かる検査メニュー「ヘルスケア」に、その主要項目の冊子が付属のセットパッケージ

「がんパック」の内容を全て含んでいます

【セット割引対象商品】詳しく見る›

On-going Project Collaborators

Academics:

- IMERI FKUI
- Lab terpadu FKUI
- Mikrobiologi FKUI
- Parasitologi FKUI
- Pulmonologi FKUI
- IKA FKUI
- IPB
- UNHAS
- UMJ

Healthcare/Govt:

- RS Persahabatan
- RSCM
- RSUI
- RS Dharmais (?)
- PJNHK (?)
- BPS
- Kemkominfo
- Kemristekdikti
- Kemenkes

Private/Sponsors:

- SATREPS/JICA
- PT Integrindos
- IDI
- IBBS
- NGOs
- PDPI(?)

Take home message

1. **On-going trend:** data & information explosion → concern in data management (mining, transfer, interoperability, storage, analysis, presentation, security) → **start small: adequate data management**
2. Main objective: BPSG profile → BPSG marker → drug discovery, preventive, etc
3. Translational medicine + data connectivity = (patient oriented) ~~personalized~~ precision medicine → backbone of medicine in Industry 4.0
4. More awareness, more exposure, more support → more knowledge, more pressure, more feasible

Adapt or perish, now as ever, is nature's inexorable imperative.

(H. G. Wells)

Thank
You
for
Your
Kind
attention