Use of pyrosequencing in clinical microbiology laboratory

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Joint Graduate Seminar Dec 2014

Department of Microbiology,

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The Chinese University of Hong Kong

Content

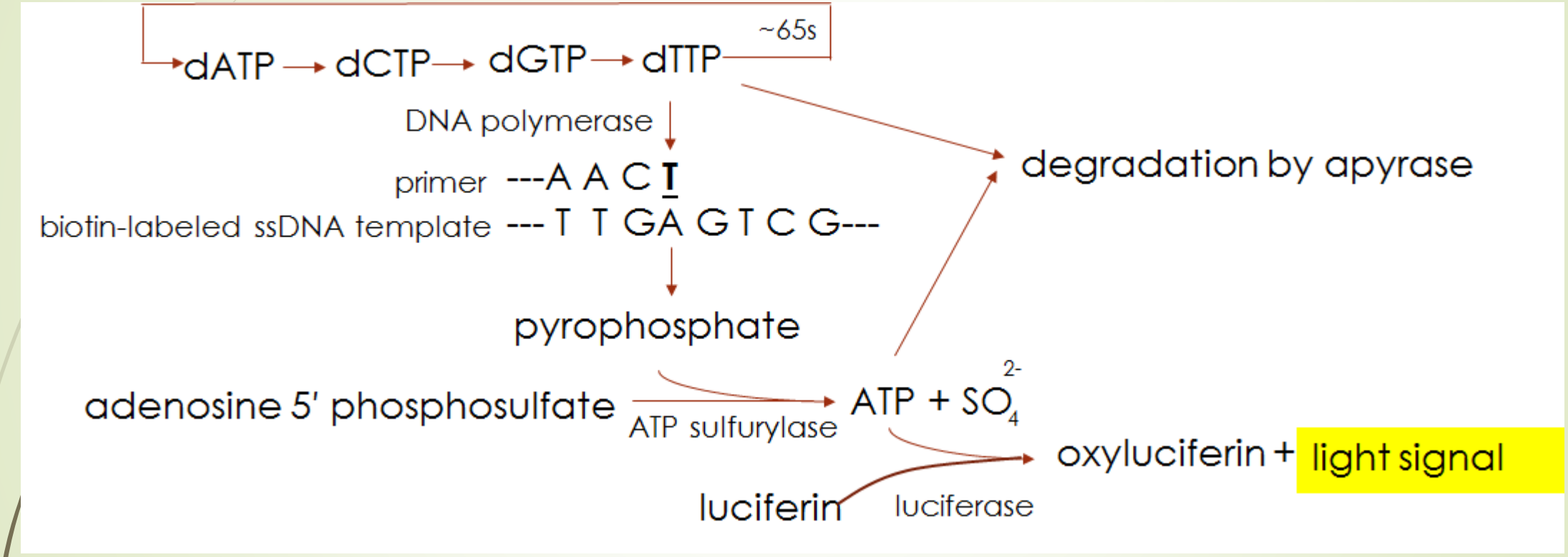
- Introduction
- Pros & Cons
- Applications of pyrosequencing in clinical microbiology lab
- Future applications
- Conclusion

DNA sequencing

- DNA sequencing is one of the most important tools for the study of microbiology today.
- Sequence determination is first performed using the chain termination sequencing method, Sanger sequencing, developed by Frederick Sanger and his colleagues in 1977 (Sanger et al., 1977) [still is in use after more than 30 years].
- Pyrosequencing, a rather “new” DNA sequencing technology, is developed by Mostafa Ronaghi and Pål Nyrén at the Royal Institute of Technology in 1996.

Pyrosequencing

- Pyrosequencing is a method of DNA sequencing based on the "**sequencing by synthesis**" principle
- Differs from Sanger sequencing, pyrosequencing relies on the detection of pyrophosphate release on nucleotide incorporation.


dATP
dCTP
dGTP
dTTP
~65s
DNA polymerase
primer ---A A C T
biotin-labeled ssDNA template --- T T GA G T C G---
pyrophosphate
degradation by apyrase
adenosine 5' phosphosulfate
ATP sulfurylase
ATP + SO4 2-
oxyluciferin +
light signal
luciferin
luciferase

Pros & Cons

Pros	Cons
1. fast (1day)	1. expensive
2. high thoroughput (>200,000 reads)	2. relatively high error rate (0.0098)
3. >200bp	3. homopolymer Ts->bad signal (more than 3-4)
4. unlimited sample number	
5. frequency data	
6. generate sequence signals immediately downstream of the primer	
7. sample preparation is easy and rapid	
8. fewer DNA templates needed	

Application-Bacterial 1

- **Bacterial identification**

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DNA Pyrosequencing-Based Bacterial Pathogen Identification in a Pediatric Hospital Setting▾†

Ruth Ann Luna[1,2], Lea R. Fasciano[1], Shaunte C. Jones[1], Bobby L. Boyanton Jr.[2], Trang T. Ton[1] and James Versalovic[1,2,*]

– Author Affiliations

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J. Clin. Microbiol. September 2007 vol. 45 no. 9 2985-2992

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Current Issue
December 2014, Volume 52, Issue 12

Luna *et al.* 2007

- DNA pyrosequencing: identification of atypical clinical isolates
- isolates that lacked a definitive identification by biochemical testing
- in a large children's hospital (Texas, USA)
- 16S rRNA genes: target sequences flanking the variable V1 and V3 regions
- 414 isolates from 312 pediatric patients
- genus- or species-level identifications: ~90% of cases

Application-Bacterial 2

- Detection of mutations that confer **antibiotic resistance**

ELSEVIER

International Journal of Antimicrobial Agents

Volume 34, Issue 5, November 2009, Pages 414–418

Detection of point mutations associated with antibiotic resistance in *Pseudomonas aeruginosa*

Neda Gorgani[a, b, 1], Scott Ahlbrand[c, 1], Andrew Patterson[c], Nader Pourmand[a, d]

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doi:10.1016/j.ijantimicag.2009.05.013

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Abstract

Excessive use of broad-spectrum antibiotics in hospitals has led to the emergence of highly resistant strains of *Pseudomonasaeruginosa*. To reduce the selection pressure for resistance, it is important to determine the antibiotic susceptibility pattern of bacteria so that hospital patients can be treated with more narrow-spectrum and target-specific antibiotics. This study describes the development of a technique for detecting point muations in the fluoroquinolone resistance-determining region of the *gyrA* and *parC* genes as well as the efflux regulatory genes *mexR*, *mexZ* and *mexOZ* that are associated with fluoroquinolone and aminoglycoside resistance. The assay is based on a short DNA sequencing method using multiplex-fast polymerase chain reaction (PCR) and Pyrosequencing™ for amplification and sequencing of the selected

Gorgani *et al.* 2009

- detect **point mutations** in 59 clinical isolates of *P. aeruginosa*

  -fluoroquinolone resistance-determining region of the *gyrA* and *parC* genes

  -efflux regulatory genes *mexR*, *mexZ* and *mexOZ* (associated with fluoroquinolone and aminoglycoside resistance)
- multiplex polymerase chain reaction
- and then pyrosequencing for sequencing of the selected genes
- Mutations related to antibiotic resistance were detected in codons 83 and 87 of *gyrA* and codon 126 of the *mexR* regulatory gene
- determine the antibiotic resistance pattern of a given bacterial strain in <1 h.

Application-Fungal 1

- **Fungal** identification

Mycoses > Vol 47 Issue 1-2 > Abstract

mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Identification of medically important fungi by the Pyrosequencing™ technology

B. Gharizadeh[1], E. Norberg[2], J. Löffler[3], S. Jalal[4], J. Tollemar[3], H. Einsele[3], L. Klingspor[2,4] and P. Nyrén[1]

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DOI: 10.1046/j.1439-0507.2003.00949.x

Issue

Mycoses
Volume 47, Issue 1-2, pages 29–33, February 2004

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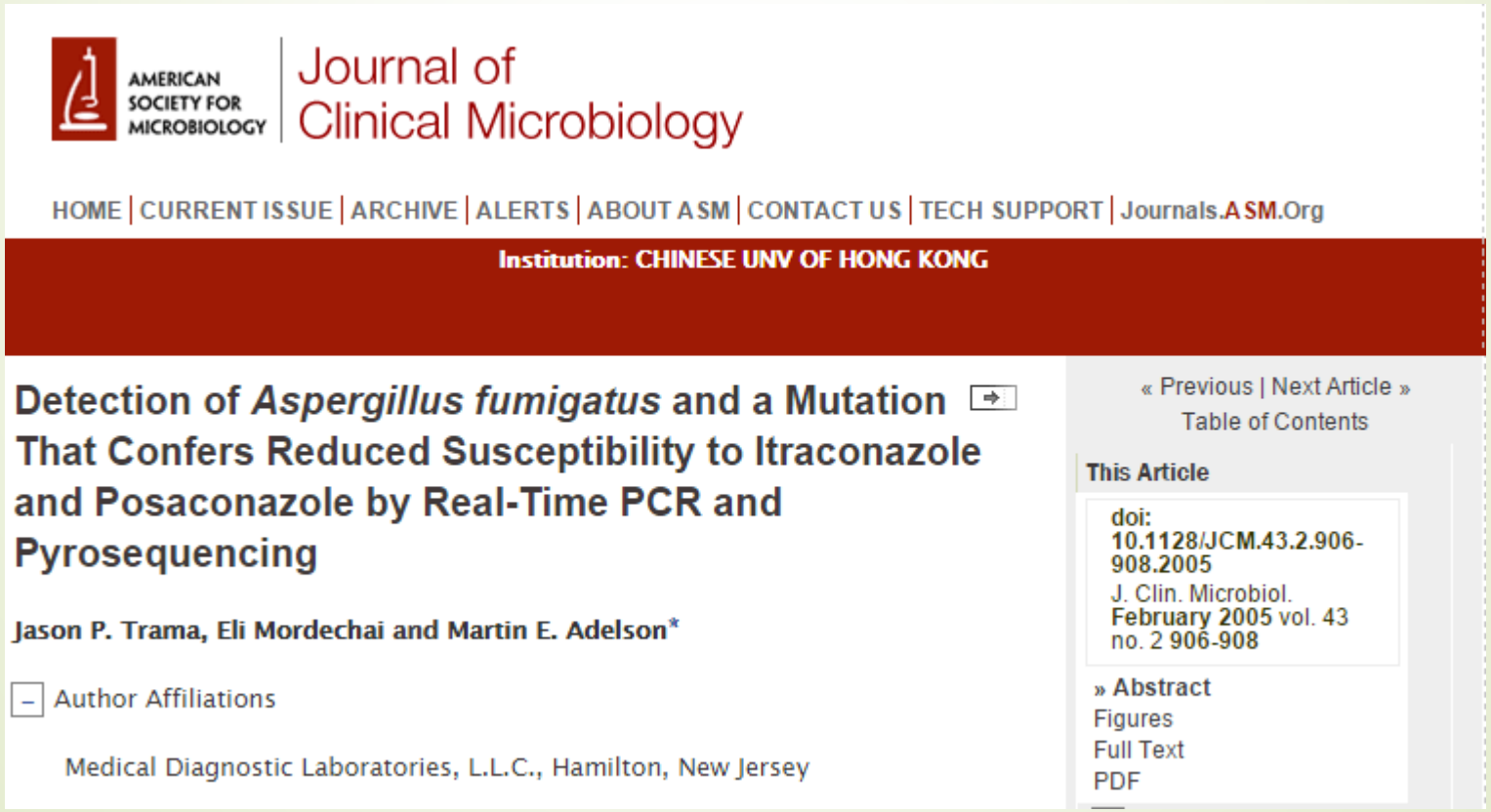
Gharizadeh *et al.* 2004

- identification of different clinically relevant fungi
- 21 fungal specimens consisting of nine strains of clinically relevant fungi
- **18S rRNA** gene using polymerase chain reaction (PCR) universal primers for amplification
- Sequencing : up to **40 bases**
- Results: all identifed
- a reproducible and reliable technique for identification of fungal pathogens.

Application-Fungal 2

- Detect mutation that confer **antifungal resistance**



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Detection of *Aspergillus fumigatus* and a Mutation That Confers Reduced Susceptibility to Itraconazole and Posaconazole by Real-Time PCR and Pyrosequencing

Jason P. Trama, Eli Mordechai and Martin E. Adelson*

Author Affiliations

Medical Diagnostic Laboratories, L.L.C., Hamilton, New Jersey

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J. Clin. Microbiol. **February 2005** vol. 43 no. 2 **906-908**

» **Abstract**
Figures
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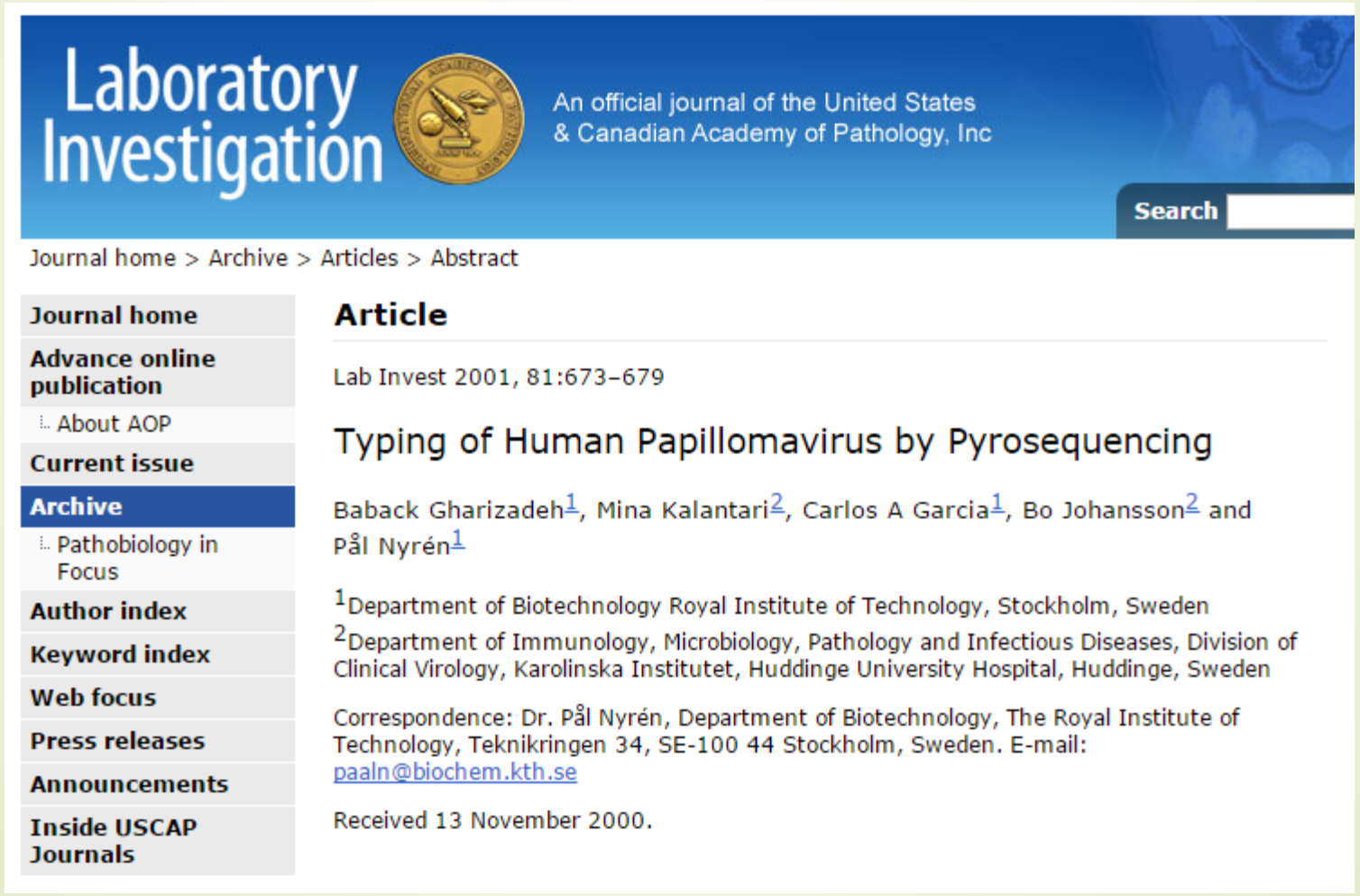
Trama *et al.* 2005

- real-time PCR and pyrosequencing
- detect *Aspergillus fumigatus* in whole blood
- *cyp51A* gene and sequencing the codon for glycine 54
- mutation: confer reduced susceptibility to itraconazole and posaconazole

Application-Viral 1

- **Viral typing**



Laboratory Investigation
An official journal of the United States & Canadian Academy of Pathology, Inc

Article

Lab Invest 2001, 81:673–679

Typing of Human Papillomavirus by Pyrosequencing

Baback Gharizadeh[1], Mina Kalantari[2], Carlos A Garcia[1], Bo Johansson[2] and Pål Nyrén[1]

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Received 13 November 2000.

Gharizadeh *et al.* 2001

- HPV genotyping by pyrosequencing
- Sequencing target: 50 nucleotide bases of the **L1 protein** gene
- Only 14 -38 bases needed

Application-Viral 2

- Monitoring antiviral resistance

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Monitoring Resistance to Human Immunodeficiency Virus Type 1 Protease Inhibitors by Pyrosequencing

Deirdre O'Meara[1], Karin Wilbe[2], Thomas Leitner[2], Bo Hejdeman[3], Jan Albert[4], and Joakim Lundeberg[1,*]

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J. Clin. Microbiol. February 2001 vol. 39 no. 2 464-473

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O'Meara *et al.* 2001

- codon changes that involved in HIV type 1 protease inhibitor resistance
- viral RNA prepared from plasma samples from HIV-1-infected individuals
- 12 primers for 34 codon changes that involved in drug resistance
- parallel analysis of 96 reactions in 1 h
- monitor drug resistance in 8 patients simultaneously

Other applications in Medicine

The Journal of Molecular Diagnostics
Volume 9, Issue 4, September 2007, Pages 464–471

Regular Articles

Application of a *BRAF* Pyrosequencing Assay for Mutation Detection and Copy Number Analysis in Malignant Melanoma

Cynthia Spittle*, M. Renee Ward†, Katherine L. Nathanson‡, Phyllis A. Gimotty§, Eric Rappaport¶, Marcia S. Brose‖, Angelica Medina‡, Richard Letrero‡, Meenhard Herlyn**, Robin H. Edwards††

* Fox Chase Cancer Center, Philadelphia, Pennsylvania
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‖ Department of Otorhinolaryngology, University of Pennsylvania, Philadelphia, Pennsylv
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¶ The Joseph Stokes Jr. Research Institute, Children's Hospital of Philadelphia, Philadelp
** The Wistar Institute, Philadelphia, Pennsylvania
† Pharmion Corporation, San Francisco, California

Accepted 23 March 2007, Available online 28 December 2010

Clinical Chemistry

Pyrosequencing Method for Genotyping Cytochrome P450 CYP2C8 and CYP2C9 Enzymes

Matthew W. Hruska, Reginald F. Frye and Taimour Y. Langaee[a]

- Author Affiliations

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In the future

- pyrosequencing more affordable, rapid, and simple to use

- Whole genome sequencing
- e.g. the genomes (common human pathogens such as *E. coli*, *Pseudomonas aeruginosa*, and *S. aureus*) range 2–5Mb
- cost of pyrosequencing: $1 to $60/Mb
- cost of sequencing a single bacterial genome:$2 to $300
- $200 to $400 per genome (included DNA preparation, etc) (Fakruddin *et al.* 2012)

Next steps:

- Microbiome analysis
  - -e.g. complex biodiversity of human guts
  - -any altered microbiome?
- Taxonomy and epidemiology
  - -novel species or subspecies
- Virtual resistance testing

Conclusion

- Pyrosequencing is a powerful tool for bacterial identification, fungal identification and viral typing in clinical microbiology laboratory
- It can also be used to detect mutations that are involved in drug resistance

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Thank you!