Molecular Detection of Resistance in Real-Time for STI Pathogens – Dawn of a New Era?

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Disclosure

Research funding from

SpeeDx

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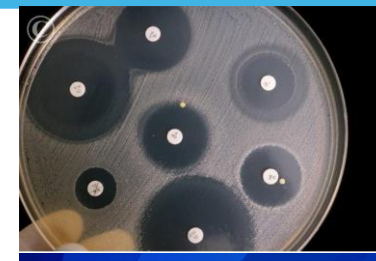
Resistance in STI Pathogens

- Antimicrobial Resistance (AMR) in STI pathogens has been around since the introduction of antimicrobial agents
  - *N.gonorrhoeae*
    - 1935: Sulphonamide treatment– resistance common by 1944
    - 1943: Penicillin available – resistance reported 1946 but continued to be used at higher doses for 40 years

Unemo M, Shafer WM. *Clinical Microbiology Reviews*. 2014;27(3):587-613..
Lewis DA. Sex Transm Infect 2010;86:415–21

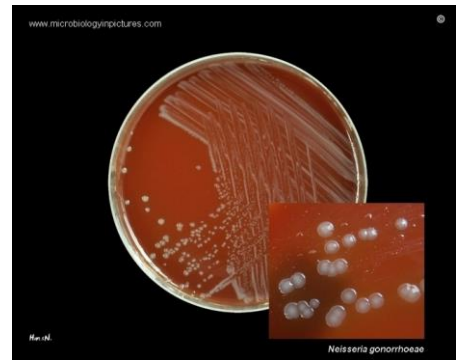
Traditional Resistance detection

- Culture – subculture = isolate
- Disk diffusion, E test
- Agar/ Broth dilution (manual, automated)
- Identification of Minimum Inhibitory Concentration (MIC)
- Organism identified as Susceptible or Resistant with reference to CLSI or EUCAST breakpoints.



Limitations of traditional methods

- Slow!
- Requires growth of organism
  - *T.p.pallidum*
  - *M.genitalium*
- Limited sensitivity of detection method



Consequences

- Doctors cannot wait and patients prescribed ineffective antibiotics
- Selection of resistant organisms
- Treatment failures & spread of disease
- Lack of faith in medical community – patients do not return for review
- Need for more expensive treatment regimes
- Untreatable organisms

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How do we stop this??

'*rapid and point-of-care diagnostic technologies are needed to reduce inappropriate and unnecessary antibiotic use*'

Responding to the threat of antimicrobial resistance: Australia's First National Antimicrobial Resistance Strategy 2015–2019

- Better and quicker diagnosis of infection and AMR
- Ideally diagnosis & resistance determined concurrently.

Real time molecular detection of resistance

- Direct from clinical samples
  - Difficult to grow organisms
- Targeted to genes of interest
  - Specific for detection and resistance determinants
  - multiple targets
- Sensitive – resistant quasi species
- Quick

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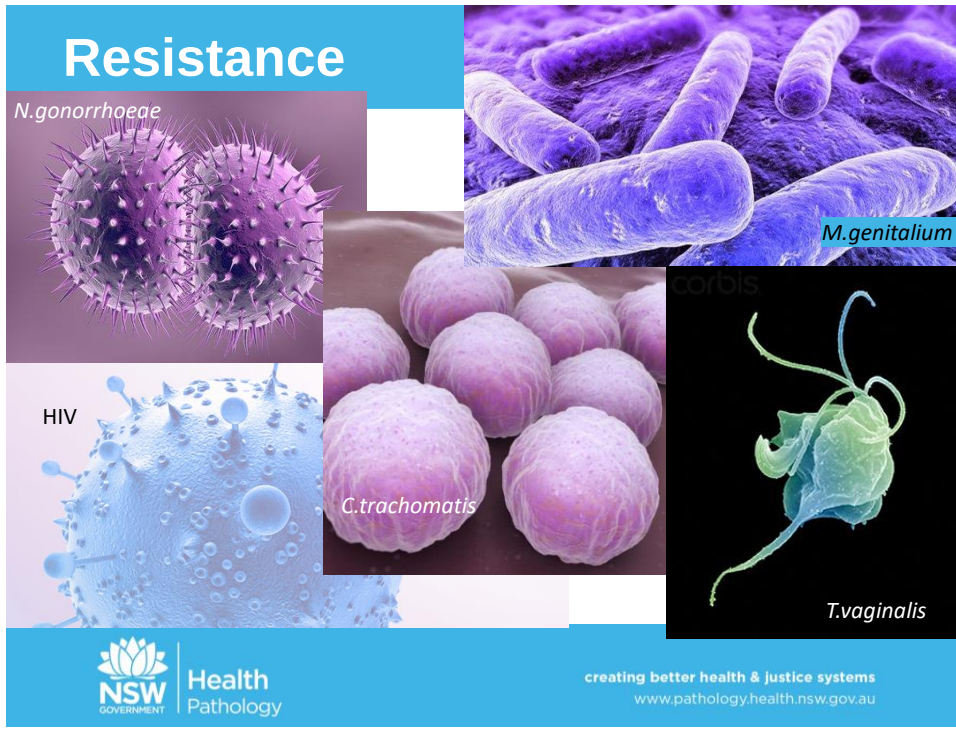

HIV Drug Resistance

- In 2016-2030 in Sub Saharan Africa – HIV DR is modelled to be responsible for
  - 16% of AIDS deaths (890 000 deaths),
    - 9% of new infections (450 000)
  - 8% ($6.5 billion) of ART program costs

Phillips AN, et al *The Journal of Infectious Diseases*. 2017.

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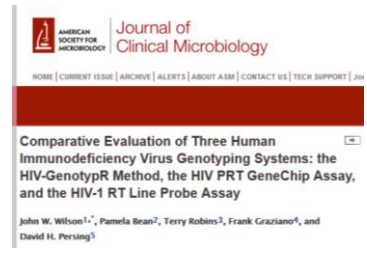
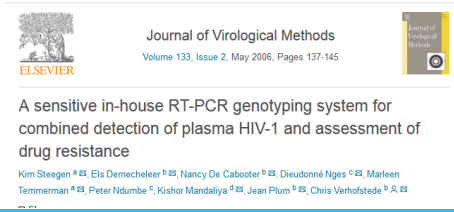
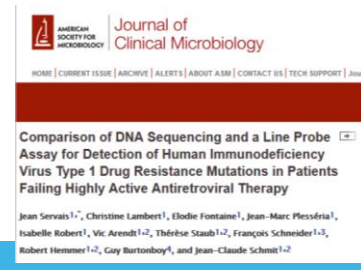
HIV Resistance Detection

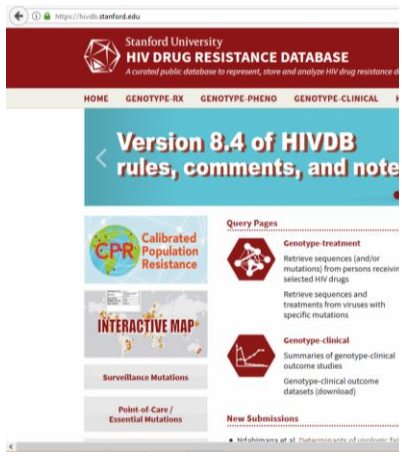
- Protease, Reverse transcriptase, Integrase genes
- In house/ Commercial
- Line probe/Chip assays
- PCR & Sanger sequencing

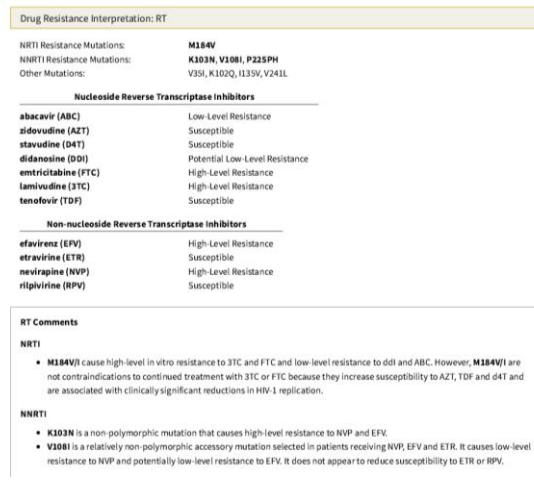






HIV Resistance databases





Resistance Testing – best practise

ashm
Antiretroviral Guidelines
US DHHS Guidelines with Australian commentary

- HIV drug-resistance testing is recommended for persons with HIV infection at entry into care to guide selection of the initial antiretroviral therapy (ART) regimen.
- HIV drug-resistance testing should be performed to assist in the selection of active drugs when changing ART regimens
- Genotypic testing is recommended as the preferred resistance testing to guide therapy in patients with suboptimal virologic response or virologic failure while on first- or second-line regimens

N.gonorrhoeae Resistance

- '*Treatment failure to the last resort of medicine for gonorrhoea (third generation cephalosporin antibiotics) has been confirmed in at least 10 countries (Australia, Austria, Canada, France, Japan, Norway, Slovenia, South Africa, Sweden and the United Kingdom of Great Britain and Northern Ireland).'* WHO -Antimicrobial resistance fact sheet (October 2017)

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NG Resistance Detection

- Culture – Gold standard
- Information limited
  - Increasing use of NAAT methods for diagnosis
  - 0.1% cases characterised worldwide
- Australian Gonococcal Surveillance Program
  - Over 30% of all Australian cases characterised by culture.
  - 10% of cases from remote areas.

Courtesy of A/Prof David Whiley, Pathology Queensland & The University of Queensland, UQ Centre for Clinical Research

NG Molecular Detection of Resistance

- To develop and apply molecular AMR methods for direct detection of *N. gonorrhoeae* resistance.
- Specific focus:
- To enhance surveillance, particularly in remote settings.
- To explore other treatment strategies.

Courtesy of A/Prof David Whiley, Pathology Queensland & The University of Queensland, UQ Centre for Clinical Research

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New molecular methods

PPNG-PCR

- PCR to detect penicillinase-producing *N.gonorrhoeae* (PPNG)
- Routine use in NT and WA where penicillin still used for therapy

Penicillin susceptibility PCR

- Used in conjunction with PPNG-PCR
- Ability to facilitate individualised treatment of gonorrhoeae with penicillin

Goire N et al J Clin Microbiol. 2011;49:513–8.
Buckley C et al. J Clin Microbiol. 2015;53:2706–8.
Speers DJ, et al. J Antimicrob Chemother. 2014;69:1243–7.
Buckley C, et al J Antimicrob Chemother. 2016 Nov;71(11):3090-3095..

New Study…

- Detection of Ciprofloxacin resistance in *N.gonorrhoeae (*SpeeDx*)*
- Will involve 9 clinics and associated labs in NT, Qld, NSW & Vic
- Allow individualised treatment decision-making.

- Advantages:
  - Reduced use of ceftriaxone
  - Broader use of oral treatments for gonorrhoea.
  - Potential for patient delivered partner therapy

Courtesy of A/Prof David Whiley, Pathology Queensland & The University of Queensland, UQ Centre for Clinical Research

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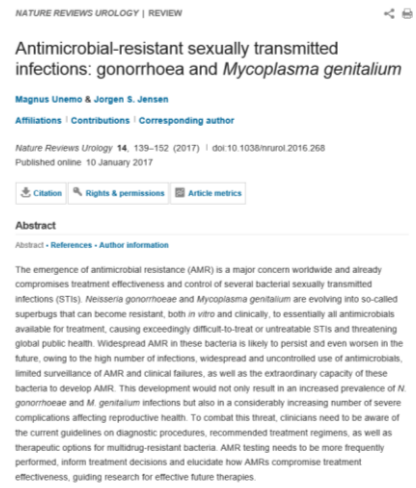
M.genitalium resistance

"*Neiserria gonorrhoeae* and *Mycoplasma genitalium* are evolving into so-called superbugs that can become resistant, both *in vitro* and clinically, to essentially all antimicrobials available for treatment, causing exceedingly difficult-to-treat or untreatable STIs and threatening global public health"

"AMR testing needs to be more frequently performed, inform treatment decisions and elucidate how AMRs compromise treatment effectiveness"



NATURE REVIEWS UROLOGY | REVIEW

Antimicrobial-resistant sexually transmitted infections: gonorrhoea and *Mycoplasma genitalium*

Magnus Unemo & Jorgen S. Jensen

Affiliations | Contributions | Corresponding author

Nature Reviews Urology **14**, 139–152 (2017) | doi:10.1038/nrurol.2016.268
Published online 10 January 2017

Citation | Rights & permissions | Article metrics

Abstract

Abstract • References • Author information

The emergence of antimicrobial resistance (AMR) is a major concern worldwide and already compromises treatment effectiveness and control of several bacterial sexually transmitted infections (STIs). *Neisseria gonorrhoeae* and *Mycoplasma genitalium* are evolving into so-called superbugs that can become resistant, both *in vitro* and clinically, to essentially all antimicrobials available for treatment, causing exceedingly difficult-to-treat or untreatable STIs and threatening global public health. Widespread AMR in these bacteria is likely to persist and even worsen in the future, owing to the high number of infections, widespread and uncontrolled use of antimicrobials, limited surveillance of AMR and clinical failures, as well as the extraordinary capacity of these bacteria to develop AMR. This development would not only result in an increased prevalence of *N. gonorrhoeae* and *M. genitalium* infections but also in a considerably increasing number of severe complications affecting reproductive health. To combat this threat, clinicians need to be aware of the current guidelines on diagnostic procedures, recommended treatment regimens, as well as therapeutic options for multidrug-resistant bacteria. AMR testing needs to be more frequently performed, inform treatment decisions and elucidate how AMRs compromise treatment effectiveness, guiding research for effective future therapies.

M.genitalium Resistance

Macrolide Resistance

- 30-100% resistance in countries where 1g AZM used routinely for NGU
- Australian Studies - Sydney study (2008-2011) - 43% contained mutations
- Melbourne study (2012-13) - 39% failed 1g AZM

Fluoroquinolone Resistance

- Sydney study (2008-2011)- 15% contained potential resistance causing mutations
- Melbourne study (2012-13) - 12% failed Moxifloxacin therapy
- First treatment failure reported in Sydney in 2013
- Pristinamycin Resistance
- Promising but treatment failures already identified

Jensen JS & Bradshaw C. *BMC Inf Dis*. 2015.
Tagg KA. et al J *Clin Microbiol* 2013;
Bissessor M et al. *Clin Infect Dis* 2015
Couldwell DL. et al *Int J STD & AIDS* 2013
Lewis D – personal communication 2017

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M.genitalium Resistance testing

- MG difficult to culture
- Diagnosis by NAAT – limited cultures available

- Molecular resistance detection increasing
- In-house/Commercial (macrolides)
- Fluoroquinolones – research only

Current studies…

- ResistancePlus MG assay (SpeeDx Pty Ltd, Sydney)
- ICPMR: Prevalence of *M.genitalium* and macrolide resistance mutations in:
  - MSM attending WSSHC (Debbie Couldwell - Wednesday 2:15pm)
  - MSM presenting with urethritis to public sexual health clinics in Sydney.
- Aim to introduce this assay into routine use

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Current studies..

- 2016/2017 Melbourne
- Prospective study - 1089 urine and anogenital swab samples
- *M. genitalium* detection – sensitivity 98.5% and specificity 100%
- Macrolide resistance mutations - sensitivity 100.0% and specificity 96.2%
- Test is now in routine use

Tabrizi et al SN PLOS ONE 2016;
Tabrizi SN. Et al J Clin Micro 2017

Aim: Individualised treatment for patients with MG
Fluoroquinolone resistance testing next on the horizon

C.trachomatis Resistance & Testing

- WHO updated treatment recommendations
  - Azithromycin, doxycycline
  - Tetracycline, erythromycin
- Treatment failures reported for tetracyclines and macrolides
- Further work being performed to identify genetic mechanisms
- Potential for real time molecular resistance testing in the future

Somani J et al– J Inf Dis 2000
Kong & Hocking – BMC Inf Dis 2009

T.vaginalis Resistance & Testing

- Most common non viral STI ~283 million cases/yr
- Treatment: metronidazole
- Resistance found in a number of clinical cases
- Further work being performed to identify genetic mechanisms
- The next molecular test needed?

Bradic M et al - Genome Biol Evol 2017
Dunn RL et al– Cell Res 2003

Current test limitations

- Current methods require knowledge of gene targets
- Possible to miss new mechanisms
- Minor changes in resistance genes can cause test to fail
- Genotypic not phenotypic – presence does not always correlate with expression
- Ideally – combination of NAAT and culture methods

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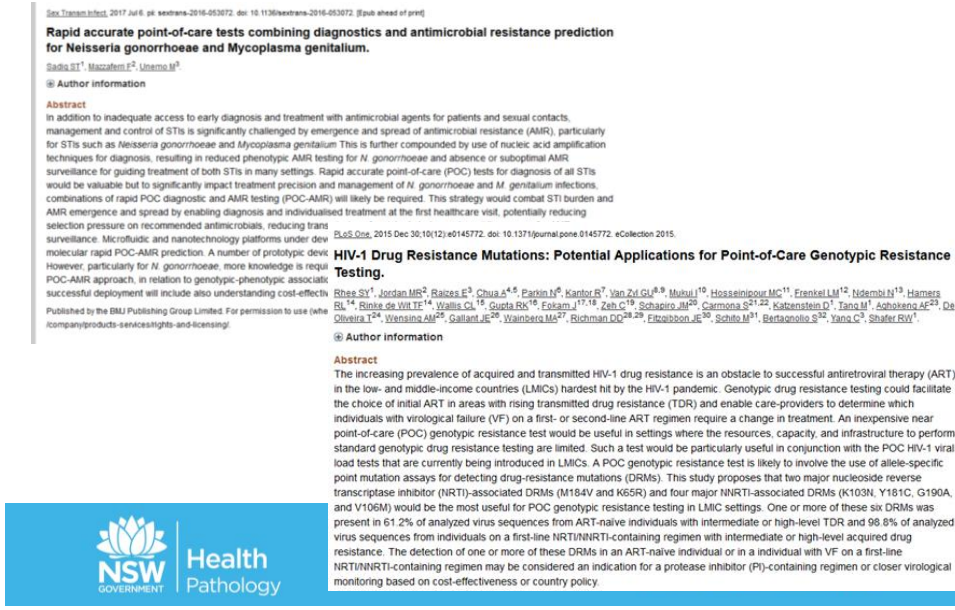
The faster the better…POC-AMR



Sex Transm Infect. 2017 Jul 6. pii: sextrans-2016-053072. doi: 10.1136/sextrans-2016-053072. [Epub ahead of print]

Rapid accurate point-of-care tests combining diagnostics and antimicrobial resistance prediction for Neisseria gonorrhoeae and Mycoplasma genitalium.

Sadiq ST[1], Mazzaferri F[2], Unemo M[3].

Author information

Abstract

in addition to inadequate access to early diagnosis and treatment with antimicrobial agents for patients and sexual contacts, management and control of STIs is significantly challenged by emergence and spread of antimicrobial resistance (AMR), particularly for STIs such as *Neisseria gonorrhoeae* and *Mycoplasma genitalium* This is further compounded by use of nucleic acid amplification techniques for diagnosis, resulting in reduced phenotypic AMR testing for *N. gonorrhoeae* and absence or suboptimal AMR surveillance for guiding treatment of both STIs in many settings. Rapid accurate point-of-care (POC) tests for diagnosis of all STIs would be valuable but to significantly impact treatment precision and management of *N. gonorrhoeae* and *M. genitalium* infections, combinations of rapid POC diagnostic and AMR testing (POC-AMR) will likely be required. This strategy would combat STI burden and AMR emergence and spread by enabling diagnosis and individualised treatment at the first healthcare visit, potentially reducing selection pressure on recommended antimicrobials, reducing trans surveillance. Microfluidic and nanotechnology platforms under dev molecular rapid POC-AMR prediction. A number of prototypic devi However, particularly for *N. gonorrhoeae*, more knowledge is requi POC-AMR approach, in relation to genotypic-phenotypic associatio successful deployment will include also understanding cost-effecti

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PLoS One. 2015 Dec 30;10(12):e0145772. doi: 10.1371/journal.pone.0145772. eCollection 2015.

HIV-1 Drug Resistance Mutations: Potential Applications for Point-of-Care Genotypic Resistance Testing.

Rhee SY[1], Jordan MR[2], Raizes E[3], Chua A[4,5], Parkin N[6], Kantor R[7], Van Zyl GU[8,9], Mukui I[10], Hosseinipour MC[11], Frenkel LM[12], Ndembi N[13], Hamers RL[14], Rinke de Wit TF[14], Wallis CL[15], Gupta RK[16], Fokam J[17,18], Zeh C[19], Schapiro JM[20], Carmona S[21,22], Katzenstein D[1], Tang M[1], Aghokeng AF[23], De Oliveira T[24], Wensing AM[25], Gallant JE[26], Wainberg MA[27], Richman DD[28,29], Fitzgibbon JE[30], Schito M[31], Bertagnolio S[32], Yang C[3], Shafer RW[1].

Author information

Abstract

The increasing prevalence of acquired and transmitted HIV-1 drug resistance is an obstacle to successful antiretroviral therapy (ART) in the low- and middle-income countries (LMICs) hardest hit by the HIV-1 pandemic. Genotypic drug resistance testing could facilitate the choice of initial ART in areas with rising transmitted drug resistance (TDR) and enable care-providers to determine which individuals with virological failure (VF) on a first- or second-line ART regimen require a change in treatment. An inexpensive near point-of-care (POC) genotypic resistance test would be useful in settings where the resources, capacity, and infrastructure to perform standard genotypic drug resistance testing are limited. Such a test would be particularly useful in conjunction with the POC HIV-1 viral load tests that are currently being introduced in LMICs. A POC genotypic resistance test is likely to involve the use of allele-specific point mutation assays for detecting drug-resistance mutations (DRMs). This study proposes that two major nucleoside reverse transcriptase inhibitor (NRTI)-associated DRMs (M184V and K65R) and four major NNRTI-associated DRMs (K103N, Y181C, G190A, and V106M) would be the most useful for POC genotypic resistance testing in LMIC settings. One or more of these six DRMs was present in 61.2% of analyzed virus sequences from ART-naïve individuals with intermediate or high-level TDR and 98.8% of analyzed virus sequences from individuals on a first-line NRTI/NNRTI-containing regimen with intermediate or high-level acquired drug resistance. The detection of one or more of these DRMs in an ART-naïve individual or in a individual with VF on a first-line NRTI/NNRTI-containing regimen may be considered an indication for a protease inhibitor (PI)-containing regimen or closer virological monitoring based on cost-effectiveness or country policy.

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Future.. Next Generation Sequencing

- Identification of all potential resistance genes (known and previously unknown)
- Development of CARDS database
- Becoming cheaper and quicker
- Potential for routine diagnostic use in the future

J Clin Microbiol. 2017 Oct 11. pii: JCM.01069-17. doi: 10.1128/JCM.01069-17. [Epub ahead of print]

Rapid Nanopore Sequencing of Plasmids and Resistance Gene Detection in Clinical Isolates.

Lemon JK[1], Khil PP[1], Frank KM[1], Dekker JP[2].

Journal List › Antimicrob Agents Chemother › v.57(7); 2013 Jul › PMC3697360

Antimicrobial Agents and Chemotherapy

AAC Article | Journal Info. | Authors | Reviewers | Permissions | Journals.ASM.org

Antimicrob Agents Chemother. 2013 Jul; 57(7): 3348–3357. PMCID: PMC3697360

doi: 10.1128/AAC.00419-13

The Comprehensive Antibiotic Resistance Database

Andrew G. McArthur,[a] Nicholas Waglechner,[a] Fazmin Nizam,[a] Austin Yan,[a] Marisa A. Azad,[a] Alison J. Baylay,[c] Kirandeep Dhullar,[a] Marc J. Canova,[a] Gianfranco De Pascale,[a] Linda Ejim,[a] Lindsay Kalan,[a] Andrew M. King,[a] Kalinka Koteva,[a] Mariya Morar,[a] Michael R. Mulvey,[b] Jonathan S. O'Brien,[a] Andrew C. Pawlowski,[a] Laura J. V. Piddock,[c] Peter Spanogiannopoulos,[a] Arlene D. Sutherland,[a] Irene Tang,[a] Patricia L. Taylor,[a] Maulik Thaker,[a] Wenliang Wang,[a] Marie Yan,[a] Tennison Yu,[a] and Gerard D. Wright[a]

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Conclusions

- AMR is an urgent global health crisis which has a significant impact on STIs and their treatment
- New methods are being developed to allow simultaneous detection of infection and identification of resistance markers
- Use of these methods will guide treatment decisions for STIs in the future to improve patient outcomes.

Acknowledgements

- Dean Jalocon ICPMR, NSWHP
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- Dr David Lewis WSSHC
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- SpeeDx
- NSW Health Pathology

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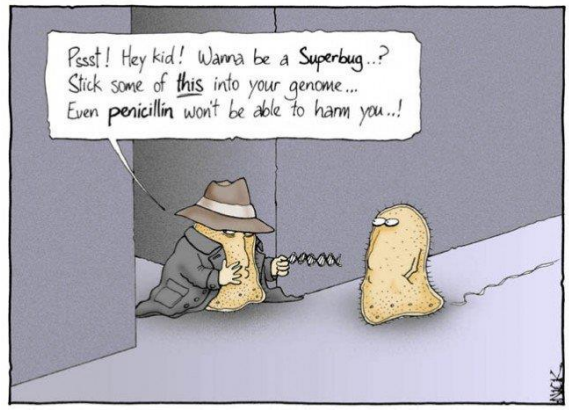

It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.