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Please contact ASHM HIV Project Officer May Wang at education@ashm.org.au if you have any enquiries.

Mycoplasma Genitalium

Meena Chandra
Registrar SSHC
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Objectives

- discuss the prevalence of *M.genitalium* in an Australian hospital setting
- overview the mechanisms of MGen resistance
- review global resistance patterns and treatment options
- look at MGen resistance in two studies done in the UK and NZ
- review resistance guided treatment

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Search Strategy

pub med, cochrane review, google scholar

Keywords : *Mycoplasma genitalium,* '*Mycoplasma genitalium'* in combination with 'resistance', 'multi-drug resistance', 'treatment', 'epidemiology', 'treatment with moxifloxacin' 'treatment with fluroquinolones'

time period :

march 2019- april 2020

Mycoplasma Genitalium

- Cause of urethritis in men
- Associated with cervicitis, PID, pre-term delivery, spontaneous abortion, ? infertility
- Asymptomatic presentations, significance unclear ?
- Sequelae and natural history likened to CT

Mycoplasma Genitalium

- 1-2% prevalence general population, similar to CT
- smallest known bacterial genome, closely related on of *Mycoplasma pneumoniae*
- emerging resistance concerning
- resistance levels vary by region and populations within an area

Should we routinely test for Mycoplasma genitalium when testing for other sexually transmitted infections? *Stewart JD et al. MJA 2020; 212 (1)*

Rationale

- Guidelines advise testing only symptomatic patients unless a contact OR, g-u surgery breaching cervical barrier
- Significance of *M. genitalium (MG)* in asymptomatic individuals, inc. pregnant women ?

Methods

- Setting : Monash medical centre (Monash Health)
- Study Design : May – July 2017, MG (PCR) test included when NG/CT requested on urine & endocervical/vaginal/urethral swabs (ED,OPD,admitted)
- Analysis/Test statistic : Mann-Whitney U-Test (ORs)

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Results and Conclusions

- ss 1176 (365m, 811f) ; median age 29y
- 56 (5%) positive MG overall; 67 (6%) positive CT
- 92/811 pregnant females, 58 were asymptomatic. 8 (9%) were MG positive however 4 of these were symptomatic
- Significant findings : age of patients for MG (younger), co-infection MG positive with CT/NG positive
- Routine testing for MG not indicated

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Mycoplasma genitalium testing results for 1176 patients at the Monash Medical Centre, Melbourne, May–July 2017

	M. genitalium testing				
	Total	Negative result	Positive result	*P**	Odds ratio (95% CI)
Number of patients	1176	1120 (95%)	56 (5%)		
Age (years), median (IQR)	29 (22–39)	29 (22–39)	24 (19–29)	< 0.001	—
Co-infection					
C. trachomatis	67 (6%)	56 (5%)	11 (20%)	—	4.6 (2.3–9.5)
N. gonorrhoeae	12 (1%)	9 (0.8%)	3 (5%)	—	7.0 (1.8–27)
Men					
Number of patients	365	353 (97%)	12 (3%)		
Age (years), median (IQR)	36 (25–50)	36 (25–50)	24 (21–35)	0.009	—
Co-infections					
C. trachomatis	20 (6%)	18 (5%)	2 (17%)	—	3.7 (0.76–18)
N. gonorrhoeae	7 (2%)	7 (2%)	0	—	—
Women					
Number of patients	811	767 (95%)	44 (5%)		
Age (years), median (IQR)	27 (20–35)	26 (20–35)	24 (19–29)	0.030	—
Pregnant	92 (11%)	84 (11%)	8 (18%)	—	1.8 (0.8–4.0)
Co-infections					
C. trachomatis	47 (6%)	36 (5%)	9 (21%)	—	5.2 (2.3–12)
N. gonorrhoeae	5 (0.06)	2 (0.3%)	3 (7%)	—	28 (4.6–172)
Pregnant women					
Number of patients	92	84 (91%)	8 (9%)		
Age (years), median (IQR)	23 (20–29)	23 (20–29)	21 (19–21)	0.28	—
Asymptomatic screen	58 (63%)	54 (64%)	4 (50%)		

CI = confidence interval; IQR = interquartile range. * Mann–Whitney *U* test.

Limitations

case series – level IV evidence

internal validity :

- limited inclusion/exclusion criteria given
-limited population demographics (gender, age, pregnancy status only)
-sexual health risk not explored

small sample of cases (4/8) were asymptomatic, unable to determine the significance of asymptomatic presentations

external validity :

-generalisability
-relevance to high risk populations
-more women (69%) than men

Molecular Basis of antimicrobial resistance in *Mycoplasma Genitalium;* *Van der Schalk TE et al. International Journal of Antimicrobial Agents (article in press 2020)*

- Aim : Review of AMR in *M. Genitalium*

- Methods :

- relevant papers searched through PubMed, ScienceDirect, and Google Scholar
- time period : December 2018, up-dated for new papers of interest.
- search items‘ *Mycoplasma genitalium* ’ alone and ‘ *Mycoplasma’* in combination with either ‘resistance’ OR ‘therapy’ OR ‘treatment’ OR ‘macrolide’ OR ‘azithromycin’ OR ‘moxifloxacin’ OR ‘doxycy- cline’ OR ‘fluoroquinolone’ OR ‘tetracycline’.

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Resistance mechanisms

- Anti-microbial treatment main mechanism of MG resistance
- Resistance dependent on SNPs (genetic variations) that occur 1/8 MGen infections
- Small genomes have higher mutation rates and less DNA repair increasing the likelihood of resistance
- High mutation rate , 10x higher *E.coli*
- **23S rRNA** mutations associated with macrolide resistance
- Fluroquinolone resistance associated with mutations in the **quinolone resistance determining region, gyrA and parC genes**

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Resistance rates against the first-line (macrolides) and second-line (fluoroquinolones) antimicrobials from recently published studies (arranged by continent)

Reference	Period of sampling (details)	Country	Resistance to	Resistance rate	Method of testing
Asia					
Kikuchi et al. [97]	2011–2013	***Japan***	Macrolides, fluoroquinolones	5/17 (29.4%) macrolides 8/17 (47.1%) fluoroquinolones	Sequencing 23S rRNA and parC genes
Deguchi et al. [70]	2013–2017 (DNA samples without strain and clinical information)	***Japan***	Macrolides, fluoroquinolones	40–70% 23S rRNA mutations 2–11% gyrA and 40–70% parC QRDR mutations	Sequencing 23S rRNA, gyrA and parC genes
Oceania					
Tagg et al. [55]	2008–2011	***Australia***	Azithromycin, moxifloxacin	62/143 (43.4%) azithromycin 23/143 (16.1%) moxifloxacin	Sequencing 23S rRNA, gyrA and parC genes
Bissessor et al. [35]	2012–2013	***Australia***	Macrolides, fluoroquinolones	56/155 (36.1%) macrolides 31/60 (51.7%) fluoroquinolones	Melt curve analysis of 23S rRNA gene, sequencing gyrA and parC genes
Murray et al. [34]	2012–2013	***Australia***	Fluoroquinolones	19/140 (13.6%) parC mutation 7/140 (5.0%) gyrA mutation 12/140 (8.6%) macrolides/fluoroquinolones combined	Sequencing 23S rRNA, gyrA and parC genes
Basu et al. [98]	2009–2015	***New Zealand***	Macrolides	86/116 (74.1%)	Sequencing 23S rRNA gene
Trevis et al. [99]	May–June 2016	***International backpackers (New Zealand)***	Macrolides	2/294 (0.7%) non-treatment-seeking volunteers [2/5 (40%) *M. genitalium*-positive patients]	SpeeDx PlexPCR™ *M. genitalium* ResistancePlus™ assay (SpeeDX Pty Ltd., Australia)
Africa					
Ong et al. [100]	2011–2012	***South Africa***	Macrolides, fluoroquinolones	0/43 specimen (0%) macrolides 19/43 specimen (44.2%) fluoroquinolones	Sequencing 23S rRNA, gyrA and parC genes
Europe					
Guschin et al. [58]	2006–2008	***Russia***	Macrolides	3/46 (6.5%)	Sequencing 23S rRNA gene
Salado-Rasmussen and Jensen [101]	2006–2010	***Denmark***	Macrolides	385/1006 (38.2%)	Sequencing 23S rRNA gene
Pond et al. [102]	2011	***UK***	Macrolides, fluoroquinolones	9/22 (40.9%) macrolides 1/22 (4.5%) fluoroquinolones	Sequencing 23S rRNA, gyrA, gyrB and parC genes
Nijhuis et al. [54]	2012–2014	***Netherlands***	Macrolides	44/146 (30.1%)	Sequencing 23S rRNA gene
Le Roy et al. [103]	2013–2014	***France***	Macrolides, fluoroquinolones	38/221 (17.2%) macrolides 12/200 (6.0%) fluoroquinolones	Sequencing 23S rRNA, gyrA and parC genes
Braam et al. [104]	2014	***Netherlands***	Macrolides	46/220 (20.9%)	Sequencing 23S rRNA gene
Dumke et al. [105]	2014–2016	***Germany***	Macrolides, fluoroquinolones	10/19 (52.6%) macrolides 2/19 (10.5%) fluoroquinolones	Sequencing 23S rRNA, gyrA and parC genes
Shipitsyna et al. [59]	2013–2016	***Russia and Estonia***	Macrolides, fluoroquinolones	44/829 (5.3%) macrolides 47/783 (6.0%) fluoroquinolones 8/766 (1.0%) macrolides/fluoroquinolones combined	Sequencing 23S rRNA and parC genes
Holynar et al. [106]	Clinical specimens for STI testing	***Finland***	Macrolides, fluoroquinolones	4/13 (30.8%) macrolides 1/13 (7.7%) fluoroquinolones	Sequencing 23S rRNA, gyrA, gyrB and parC genes
Mulligan et al. [107]	Nov. 2017–Jan. 2018	***Ireland***	Macrolides, fluoroquinolones	9/12 (75.0%) macrolides 4/12 (33.3%) fluoroquinolones	PCR of 23S rRNA and parC genes, and sequencing 23S rRNA, gyrA and parC genes
Middle East					
Maatouk [108]	Case study	***Lebanon***	Ciprofloxacin, azithromycin	1 resistant patient	Only treatments stated; patient was susceptible to moxifloxacin
North America					
Chernesky et al. [109]	Single measurement	***Canada***	Macrolides, fluoroquinolones	26/55 (47.3%) macrolides 1/53 (1.9%) fluoroquinolones	Sequencing 23S rRNA, gyrA and parC genes
Mondeja et al. [110]	2009–2016	***Cuba***	Macrolides	64/202 (31.7%) 64% resistance since 2014	5′ nuclease genotyping assay for macrolide resistance-mediating mutations

Current treatment options

1. Azithromycin :
   - treatment of CT with azithromycin contributing to resistance
   - treatment failure due to low accumulation of azithromycin in the nucleus of epithelial cells where MG localises

2. Moxifloxacin
   - 2nd line treatment, growing resistance

3. Pristinomycin, spectinomycin
   - 3rd line treatment, rescue therapy for DTR, limited data

4. Doxycycline
   - moderately effective (31-45 % treatment efficacy with doxycycline alone)

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fluroquinolones

- Bind to 2 targets DNA gyrase and DNA topoisomerase inducing DNA breaks with a bactericidal effect

- Due to their mechanism of action they can increase the frequency of mutations causing induction of SNPs in DNA gyrase and topoisomerase genes

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Efficacy of pristinomycin

- Comprised of 2 antibiotics – depsipeptide and ketolide (macrolide), this combination 100x more effective
- A small study in 2015 showed clearance of azithromycin resistance MG with pristinomycin
- The study however also reported SNPs in pristinomycin treated patients and so resistance cannot be excluded

novel treatment options

- spectinomycin - (aminoglycoside) effective in vivo and in vitro against macrolide resistant strains in a case report
- minocycline - (tetracycline) effective against DTR MG strains in 2 case reports
- solithromycin - (macrolide), clinical trial efficacy against CT and NG, based on in vitro studies estimated clinical cure of 65-85% against azithromycin resistance strains , no clinical trials conducted
- Zoliflodacin - in vitro studies against MDR strains of MG showed promising results

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Mycoplasma genitalium Antimicrobial Resistance in Community and Sexual Health Clinic Patients, Auckland,New Zealand.

Vesty et al. Emerging Infectious Diseases 2020; 26 (2)

- Rationale
  - Azithromycin first line treatment, fluroquinolones second line treatment in NZ for *M.genitalium*
  - 72% macrolide resistance ; 23% fluroquinolone resistance in NZ
- Aim

  -determine *M. genitalium* resistance patterns in two different population settings in NZ

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Methods

- Setting : Auckland city hospital
- Study Design : Retrospective study. DNA samples from urine (92%) or urogenital swabs testing positive for MG were retrieved for resistance testing [macrolide resistance (23S rRNA mutations); [fluoroquinolone resistance (gyrA and parC mutations)]
- Test Statistic : $\chi 2$ test

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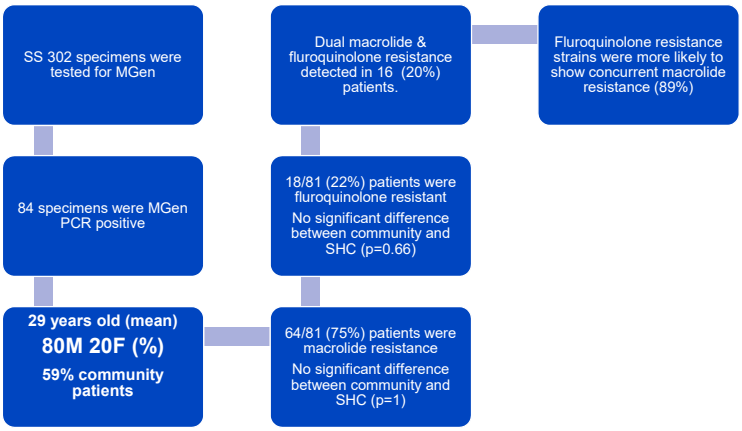
Results



Limitations

Internal validity – limited inclusion criteria, limited demographics, majority males (selection bias)

External validity – generalisability , majority males, however general and SHC patients

Clinical relevance , contact Vs cases

Unable to establish when resistance occurred

Antimicrobial resistance in *Mycoplasma genitalium* sampled from the British general population *Pitt R et al. Sex Trans infect 2020; 0(1-5)*

- Rationale :
  - -UK prevalence of MG similar to CT 16-44y
  - -higher prevalence in MSM and FSW
  - -no data on the occurrence of *M. genitalium* AMR determinants in specimens from the general population to inform policy decisions in the UK
- Aim: investigate the distribution of genotypically determined resistance in MG positive specimens from the sexually active British population

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Methods

Setting: UK general population

Recruitment and Study Design : Recruitment of participants from (Nastsal-3) 2010-12. Sample selected based on responses from CASI. Participants posted FVU to Public Health England for testing

Laboratory methods : MG (RT-PCR); macrolide and fluroquinolone resistance (23S rRNA and parC gene)

Test statistic : fishers 2-sided t-test

Results and Conclusions

- SS 4507
- 66 positive for MG
- Macrolide resistance associated with 23S rRNA gene mutation was detected in 9/56 (16%) (CI 9-28%)
- Fluroquinolone resistance associated with parC gene mutation was detected in 2/61 (3.3%) (CI 0.9-11.2 %)
- Patient characteristics with macrolide resistance
  - Hx of STI
  - SHC attendance
  - ≥ 2 sexual partners in the last year
  - Asymptomatic

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Table 2 Sociodemographic, behavioural, and clinical risk factors for macrolide resistance-conferring mutations in the 23S rRNA gene in *Mycoplasma genitalium* specimens from a sexually-active probability sample of the general British population

	M. genitalium with resistance-conferring mutation			Susceptible *M. genitalium*			Total	
	No.	%	95% CI	No		95% CI	No.	P value
	9	16.1	8.7 to 27.8	47	83.9	72.2 to 91.3	56	
Sex								
Male	3	14.3	5.0 to 34.6	18	85.7	65.4 to 95.0	21	1.000
Female	6	17.1	8.1 to 32.7	29	82.9	67.3 to 91.9	35	
Age (years)								
16–24	5	22.7	10.1 to 43.4	17	77.3	56.6 to 89.9	22	0.479
25–34	4	14.8	5.9 to 32.5	23	85.2	67.5 to 94.1	27	
35–44	0	0	–	7	100.0	64.6 to 100	7	
Ethnic group*								
White	7	15.6	7.8 to 28.8	38	84.4	71.2 to 92.3	45	0.611
Black/Black British	2	25.0	7.2 to 59.1	6	75.0	40.9 to 92.9	8	
No. sexual partners, past year†								
2+	8	25.0	13.3 to 42.1	24	75.0	57.9 to 86.8	32	0.063
0–1	1	4.2	0.7 to 20.3	23	95.8	79.8 to 99.3	24	
No. new sexual partners, past year †								
1+	8	24.2	12.8 to 41.0	25	75.7	59.0 to 87.2	33	0.067
0	1	4.4	0.8 to 21.0	22	95.7	79.0 to 99.2	23	
Unsafe sex, past year ‡								
Yes	3	21.4	7.6 to 47.6	11	78.6	52.4 to 92.4	14	0.678
No	6	14.6	6.9 to 28.4	35	85.4	72.6 to 93.1	41	
STI symptoms, past month§								
Yes	0	0	–	12	100.0	75.8 to 100	12	0.180
No/not mentioned	9	20.5	11.2 to 34.5	35	79.5	65.5 to 88.9	44	
Diagnosed with any STI, past 5 years								
Yes	4	44.4	18.9 to 73.3	5	55.6	26.7 to 81.1	9	0.029
No	5	10.6	4.6 to 22.6	42	89.4	77.4 to 95.4	47	
Sexual health clinic attendance								
Yes	9	24.3	13.4 to 40.1	28	75.7	59.9 to 86.6	37	0.021
In the last year	7	43.8	23.1 to 66.8	9	56.3	33.2 to 76.9	16	0.001
1+years ago	2	12.5	3.5 to 36.0	19	90.5	71.1 to 97.4	21	
No	0	0	–	19	100.0	83.2 to 100	19	

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Strengths and Limitations

- strengths
  - general population sample as opposed to STI diagnosed samples where selection bias may lead to overestimation of the scale of the problem
  - High proportion of specimens with sequencing data

- limitations
  - specimens stored upto 6y, re-confirmation and AMR testing
  - report only on 23S rRNA and parC gene mutations
  - patient characteristics only determined for macrolide resistance due to small ss
  - asymptomatic testing (limited data), modelling (cost-effective analysis)

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Outcomes of Resistance-guided Sequential Treatment of *Mycoplasma Genitalium* Infections: A prospective Evaluation; *Read T R H et al. Clinical Infectious Diseases 2019; 68(4)*

- Aim : To evaluate outcomes of sequential antimicrobial therapy for MG guided by macrolide resistance assay

- Rationale :

- Azithromycin treatment has led to selection (post-treatment detection) of strains with MRMs
- Not enough epidemiological data around resistance (prevalence, acquired Vs pre-existing mutations)

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METHODS

Setting : MSHC

Recruitment and Study Design :

-prospective evaluation of patients diagnosed with MG and treated with azithromycin, doxycycline as a pre-treatment, and if treatment was based on their resistance assay from 2016- 2017

-subset of patients were asked to provide another urine sample at collection of 2nd antibiotic to determine bacterial load of MG

-pre & post treatment resistance assays

-cases excluded if not treated according to their resistance result, did not return for TOC, and had a high chance of reinfection

Lab methods: diagnostic-resistance PCR assay (ResistancePlusMG, SpeeDx)

Data Analysis : univariate logistic regressions, paired t-test

Table 2. Characteristics of the Population Studied for Outcomes of *Mycoplasma genitalium* Treatment

Characteristic	Macrolide Susceptible (n = 77)	Macrolide Resistant (n = 167)	Total (N = 244)
Time to test of cure[a], d, median (IQR)	29 (25–41)	27 (22–32)	28 (22–35)
Age, y, median (IQR)	26.0 (22.9–30.9)	28.7 (25.6–34.4)	27.9 (24.5–33.0)
Sex/sexuality[b]			
Female	29 (37.7)	23 (13.8)	52 (21.3)
Male, heterosexual	32 (41.6)	36 (21.6)	68 (27.9)
MSM	16 (20.8)	108 (64.7)	124 (50.8)
Site of detection[c]			
Cervix/vagina	24 (31.1)	21 (12.6)	45 (18.4)
Urine	49 (63.6)	105 (62.9)	154 (63.1)
Rectum[d]	4 (5.2)	41 (24.6)	45 (18.4)
HIV serostatus			
Negative	62 (80.5)	141 (84.4)	203 (83.2)
Untested	15 (19.5)	10 (6.0)	25 (10.3)
Positive	0	16 (9.58)	16 (6.56)
Asymptomatic[c]	18 (23.4)	43 (25.8)	61 (25.0)
Symptomatic	59 (76.6)	124 (74.3)	183 (75.0)
Clinical diagnosis			
Nongonococcal urethritis	31 (40.3)	78 (46.7)	109 (44.7)
Contact of *Mycoplasma genitalium*	21 (27.3)	38 (22.8)	59 (24.2)
Other[e]	5 (6.5)	28 (16.8)	33 (13.5)
Vaginal discharge/bleeding	13 (16.9)	7 (4.2)	20 (8.2)
Proctitis	3 (3.9)	12 (7.2)	15 (6.2)
Cervicitis/PID	4 (5.2)	4 (2.4)	8 (3.3)

Results

- SS 244 (52W, 68M, 124 MSM)
- All patients treated with doxycycline and then azithromycin or sitofloxacin
- 77 (32%) macrolide sensitive – 73 cured
- 4 tested positive for MG at TOC, ¾ mutations detected in the post-treatment sample; ¼ MRMs pretreatment sequencing

Results- Macrolide Resistance cases

- MRMs detected in 167 cases (68%) (CI 62-74%)
- Cure in 154 (95%) (CI 87-96%) cases

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Doxcycline load

- 56 males with urethritis were tested for MG load after 7/7 doxycycline BD
- Tx with doxy reduced bacterial load by a mean 2.60 $\log_{10}$ ($p<0.0001$)
- Undectectable 39% ; reduced in 50% ; increased in 11%

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Strengths and Limitations

- Strengths :

-high cure rates for treatment protocol in a setting with high rates of AMR
-first study to demonstrate treatment efficacy in MG using this treatment protocol
-limitations of study clearly stated

- Limitations :

-internal validity, losses to follow-up, high msm (selection bias)
-unable to ascertain whether the cure rate in the macrolide sensitive study arm was due to increased dose of azithromycin, doxyclycline loading, or both
-effect of doxycycline on bacterial load
-no control group (however exclusion criteria clearly stated)
-external validity - generalisability to the general population

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Conclusions

"*M. genitalium* is an important and prevalent STI, and one where management and control is challenging because it is under-tested, under-detected, and difficult to treat"

Greater need for evidence-based third line agents like pristinomycin, minocycline, lefamulin, gepotidacin, solithromycin, zoliflodacin

Dual agent sequential therapy based on resistance assays has shown efficacy in treatment

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