

doi: 10.13241/j.cnki.pmb.2014.18.018

106 例宫颈炎患者支原体检测及抗生素敏感性分析*

李静宜¹ 金影² 姜越¹ 辛德莉^{1△}

(1 首都医科大学附属北京友谊医院 北京热带医学研究所 北京 100050; 2 首都医科大学附属北京友谊医院妇产科 北京 100050)

摘要 目的:对本地区宫颈炎患者解脲脲原体(Uu)和人型支原体(Mh)进行监测,分析抗生素敏感情况,为临床诊疗提供实验依据。**方法**:无菌采集宫颈炎患者宫颈分泌物,用支原体培养、鉴定、药敏试剂盒进行培养和药敏试验。**结果**:支原体总检出率为71.70%。Uu检出率为52.83%,Uu合并Mh检出率16.04%,Mh检出率为2.83%。56例单纯Uu株体外抗生素敏感性显示:对四环素族多西环素100%、美满霉素98.21%高度敏感;对大环内酯类敏感性不一:克拉霉素96.43%,阿奇霉素91.07%,交沙霉素76.79%,红霉素67.86%,罗红霉素32.14%;对喹诺酮类如加替沙星83.93%较敏感,而对左氧氟沙星、司帕沙星敏感性差;对克林霉素和甲砒霉素不敏感;17例Uu合并Mh的抗生素敏感性为:美满霉素100%,多西环素100%,交沙霉素88.23%,加替沙星64.7%,对左氧氟沙星、司帕沙星、红霉素、阿奇霉素、克拉霉素、罗红霉素、甲砒霉素、克林霉素均不敏感。3株单纯Mh例数少未做统计。**结论**:宫颈炎患者中支原体检出率较高,其中单纯Uu检出率较Uu、Mh混合阳性率高,对四环素类、大环内酯类族中的多数抗生素敏感,治疗选择余地大。Uu、Mh混合敏感性抗生素种类少,应优先选择四环素类抗生素治疗。

关键词:宫颈炎;支原体;药敏试验

中图分类号:R-375-1 文献标识码:A 文章编号:1673-6273(2014)18-3473-03

Analysis of Detection and Antibiotic Susceptibility Results of 106 Cases with Cervicitis*

LI Jing-yi¹, JIN Ying², JIANG Yue¹, XIN De-li^{1△}

(1 Beijing Tropical Medicine Research Institute, Beijing Friendship Hospital, Capital Medical University, Beijing, 100050, China;

2 Department of Obstetrics and Gynecology, Beijing Friendship Hospital, Capital Medical University, Beijing, 100050, China)

ABSTRACT Objective: The laboratorial data were collected for clinical diagnosis and treatment by monitoring the Ureaplasma urealyticum and Macoplasma hominis infection to evaluate the susceptibility to the antibiotics of strains isolated from cases with cervicitis in local area. **Methods:** Cervical secretions of the patients suffered with cervicitis were got sterilely. The culture, identification, susceptibility testing were carried out with complex mycoplasma kits. **Results:** In 106 specimens, there were 76 cases with mycoplasma infection, and the total isolation rate was 71.70%. The isolation rate of Uu, Uu combined Mh, and Mh was 52.83%, 16.04%, 2.83%, respectively. Susceptibility testing data showed that 56 cases of Uu positive were high sensitive to tetracycline, for example, doxycycline 100%, minocycline 98.21%; It showed different sensitivity to macrolides antibiotics: clarithromycin 96.43%, azithromycin 91.07%, josamycin 76.79%, erythrocin 67.86%, roxithromycin 32.14%; For quinolone antibiotics, the sensitivity were 83.93% to gatifloxacin, 37.50% to levofloxacin, 26.78% to sparfloxacin; Sensitivity rate to clindamycin and thiamphenicol were 0%, 5.36% respectively; The 17 cases Uu combined with Mh were susceptible to minocycline 100%, doxycycline 100%, josamycin 88.23%, gatifloxacin 64.7%, respectively. But, they were not susceptible to levofloxacin, sparfloxacin, erythrocin, azithromycin, clarithromycin, roxithromycin, thiamphenicol, clindamycin. The 3 cases with Mh were not calculated, because the number was too small. **Conclusions:** Mycoplasma isolation rate of cervical secretions collected from the patients with cervicitis was high. Uu isolation rate was higher than Uu combined with Mh, and it was susceptible to most of tetracyclines and macrolides antibiotics. Because sensible antibiotics were less, tetracyclines antibiotics should be prioritized in the treatment of patients who isolated Uu and Mh.

Key words: Cervicitis; Mycoplasma; Susceptibility testing**Chinese Library Classification:** R-375-1 **Document code:** A**Article ID:** 1673-6273(2014)18-3473-03

前言

解脲脲原体(Ureaplasma urealyticum)、人型支原体(M. ho-

minis)寄生于人泌尿生殖道,是非淋菌性宫颈炎(NGU)的主要病原体之一^[1]。有报道显示Uu非粘液性宫颈炎中阳性分离率也较高^[2]。另外Biernat-Sudolska等发现HPV阳性病例中Uu检

* 基金项目:国家自然科学基金项目(81271890);北京市支原体耐药检测项目(Z111107055311042)

作者简介:李静宜(1978-),女,硕士,助理研究员,主要研究方向:病原生物学、呼吸系统疾病

△ 通讯作者:辛德莉,电话:13718660994, E-mail: xindl48@126.com

(收稿日期:2013-11-09 接受日期:2013-11-30)

出率较高,且宫颈病患者 Uu 的感染率也高于对照组^[3]。多个研究表明各人群宫颈的 Uu 和 Mh 检出率并不一致, 抗生素敏感性差异也较大^[4-6],有明显的时空差异性。另外由于抗生素使用不规范等原因,导致支原体的耐药率日渐升高,耐药株逐年增加,尤其多重耐药菌株的增多^[7]。使得非淋菌性宫颈炎反复发作或迁延不愈,或可诱发子宫内膜炎、输卵管炎、盆腔炎,导致不孕^[8],早产^[9]等给患者带来较大痛苦和经济负担。为掌握本院宫颈炎患者支原体检出及抗生素敏感情况及耐药变化规律,向临床治疗支原体感染及合理使用抗生素提供必要的实验依据,故对本院 2012 年 6 月 -10 月中诊断为宫颈炎的患者的宫颈分泌物进行了支原体培养和药敏实验。

1 材料与方法

1.1 实验材料

1.1.1 标本来源 宫颈分泌物采集自我院 2012 年 6 月至 10 月就诊的宫颈炎患者。

1.1.2 试剂 采用郑州安图绿科生物工程有限公司提供的支原体培养鉴定计数药敏试剂盒【豫食药监械(准)字 2009 第 2400041 号】进行支原体培养鉴定及药敏实验。

1.2 检测方法

1.2.1 标本采集 严格按照全国临床检验操作规程进行标本采集。常规消毒外阴,一次性无菌窥器扩阴道暴露宫颈,先用消毒棉签擦去宫颈外口粘液及分泌物,后用专用拭子插入宫颈管内 2 cm~3 cm 处,停留 10 秒,旋转 3 次,约等 10 秒后取出。

1.2.2 培养及药敏实验 按试剂盒说明书进行支原体鉴定及药敏实验,严格遵守无菌操作规范。操作如下:支原体培养基础液及药敏板恢复至室温,吸取 100 μ L 基础液加入 C-(阴性对照孔)。采样拭子直接接种于剩余基础培养液中,摇匀。将含标本的基础液加入其余板孔(C+ 阳性对照孔,Uu,Uu $\geq 10^4$,MH,MH $\geq 10^4$,及 12 中抗生素不同浓度的板孔中,每孔 100 μ L,轻轻震荡药敏板。每孔滴加 1-2 滴矿物油,药敏板加盖至培养箱 37℃ 培养,24 小时观察结果。

1.2.3 结果判定 按说明书判读结果。24 小时候培养孔清亮且

由黄色变为桃红色判定阳性,未变色判定为阴性。相同抗生素高低浓度的上下两孔均为阴性,判定对该抗生素敏感;同种抗生素低浓度上孔阳性,高浓度下孔阴性,判定对该抗生素中度敏感;在上下两孔均呈阳性,则判定对该抗生素耐药。

2 结果

2.1 支原体检出情况

共检查疑似支原体感染宫颈炎患者 106 例,支原体检出 76 例,总检出率为 71.70%。其中单纯 Uu 56 例,占比 52.83%,Uu、Mh 混合检出 17 例,占 16.04%,单纯 Mh 检出 3 例,仅占 2.83%(表 1)。

表 1 支原体检出率

Table 1 Positive rate of mycoplasma

Category	Number	Percentage (%)
Uu	56	52.83
Uu+Mh	17	16.04
Mh	3	2.83
Negative	30	28.30
Total	106	100

2.2 体外抗生素敏感实验

56 例单纯 Uu 感染体外抗生素敏感性:对四环素类抗生素均高度敏感;对喹诺酮类抗生素如加替沙星较敏感,而对左氧氟沙星、司帕沙星敏感性差;对大环内酯类抗生素敏感性不一:对克拉霉素、阿奇霉素敏感性高,交沙霉素、红霉素次之,对罗红霉素最不敏感;对甲砒霉素和克林霉素均不敏感。17 例 Uu 合并 Mh 的对美满霉素、多西环素敏感性达 100%;其次为交沙霉素、加替沙星;对左氧氟沙星、司帕沙星、红霉素、阿奇霉素、克拉霉素、罗红霉素、甲砒霉素、克林霉素则不敏感(表 2)。3 株单纯 Mh 对美满霉素、多西环素、克林霉素和交沙霉素 100%敏感,对红霉素、阿奇霉素、罗红霉素和克拉霉素 100%耐药,对司帕沙星、加替沙星、左氧氟沙星和甲砒霉素 66.7%敏感(结果未显示)。

表 2 支原体培养阳性抗菌药物体外药物敏感情况(%)

Table 2 Antibiotics susceptible test of Mycoplasma isolated from the cervicitis patients (%)

Antimicrobial Agent	Uu			Uu+Mh		
	Susceptible (%)	Intermediate (%)	Resistance (%)	Susceptible (%)	Intermediate (%)	Resistance (%)
Minocycline	98.2	1.8	0	100	0	0
Doxycycline	100.0	0	0	100	0	0
Erythrocine	67.9	28.6	3.5	5.9	0.0	94.1
Azithromycin	91.0	5.4	3.6	5.9	5.9	88.2
Josamycin	76.8	21.4	1.8	88.2	11.8	0
Roxithromycin	32.1	62.5	5.4	5.9	5.9	88.2
Clarithromycin	96.4	0.0	3.6	5.9	5.9	88.2
Sparfloxacin	26.8	51.8	21.4	11.8	47	41.2
Gatifloxacin	83.9	14.3	1.8	64.8	29.4	5.8
Levofloxacin	37.5	58.9	3.6	11.8	76.4	11.8
Clindamycin	0	50.0	50.0	5.9	52.9	41.2
Thiamphenicol	5.3	66.0	28.6	0	76.5	23.5

3 讨论

支原体(Mycoplasma)是一类缺乏细胞壁,可在无生命培养基中生长繁殖的最小原核细胞型微生物。归属于柔膜体纲,支原体目(Mycoplasmatales),支原体科;支原体科又分为支原体属和脲原体属。目前从人体分离出的16种支原体中,7种对人有致病性。与女性宫颈炎相关支原体有三种,解脲、人型和生殖支原体^[10]。本文对解脲和人型支原体进行了调查,发现在宫颈炎患者中Uu检出率较高为52.83%,其次为Uu+Mh混合为16.04%,单纯Mh检出较少为2.83%,与韩日新等研究类似^[11]。刘朝晖对正常人群宫颈分泌物监测Uu结果显示Uu液体培养阳性60.9%,但Uu半定量大于 10^4 者仅占19.9%^[12]。本实验中在宫颈炎患者支原体定量CCU $\geq 10^4$ 者占68%,明显比正常人群高,提示支原体在体内的大量增生与宫颈炎相关,或可能引起或加重宫颈炎^[13]。

支原体因缺乏细胞壁结构,因此对作用于细胞壁的抗生素,如 β -内酰胺类、万古霉素等完全不敏感;对多黏菌素、利福平和磺胺类药物普遍耐药。对支原体最有抑制活性及常用于支原体感染治疗的抗生素是四环素类、大环内酯类及氟喹诺酮类药物^[14]。四环素类抗生素能特异性地与细菌核糖体30S亚基的A位置结合,阻止氨基酰-tRNA在该位上的联结,抑制肽链的增长和影响蛋白质合成。罗芳研究显示,武汉地区对四环素的耐药率为19.0%,对多西霉素耐药率为3.2%,对米诺环素耐药率为4.2%^[15]。本实验宫颈炎患者检出的Uu和Mh对美满霉素、多西环素敏感性为98.2%~100%,耐药率为0%。大环内酯类抗生素能不可逆的结合到核糖体50S亚基上,通过阻断转肽作用及mRNA位移,选择性抑制蛋白质合成。研究表明Uu对大环内酯类抗生素耐药主要是在23S rRNA V区和核糖体蛋白L4突变所致^[16]。Uu对阿奇霉素、克拉霉素较敏感,分别为91%、96.4%;交沙霉素次之,罗红霉素最差,分别为76.8%、32.1%。所得结论与Farka等的研究类似^[17],但与张吕良报道Uu对阿奇霉素敏感率为6.1%不同,可能是因为不同地区Uu流行型别不同,抗生素使用习惯不同所导致的^[18]。Mh对红霉素等抗生素天然耐药,因此对Mh感染的患者应不选择红霉素类抗生素。另外,本研究显示,Mh仅对大环内酯类抗生素中交沙霉素的敏感性较好。单纯Uu、Uu合并Mh、单纯Mh敏感率分别为76.8%、88.2%和100%。这可能是由于交沙霉素化学结构中的支链构造不同或临床应用较少等原因导致的^[19]。喹诺酮类抗生素是人工合成的含4-喹诺酮母核基本结构的抗菌药物,主要通过抑制DNA回旋酶及拓扑异构酶IV活性从而达到抗菌效果,目前耐药主要是拓扑异构酶IV ParC蛋白83位丝氨酸(S)到亮氨酸(L)的突变导致的^[20]。目前Uu对司帕沙星的敏感性较低,仅为26.8%,而耐药率达到21.4%;对加替沙星和左氧氟沙星耐药率低,但对左氧氟沙星的敏感性仅为37.5%。考虑因为临床上左氧氟沙星应用范围较广泛,泌尿系统、肠道感染均可以使用,疗程长,剂量大导致Uu对其敏感性下降,而其中度敏感率上升为58.9%。林可霉素类包括林可霉素及克林霉素,克林霉素为林可霉素衍生物,抗菌效果较林可霉素更强,口服吸收好且毒性较小,临床较为常用。Mh对于克林霉素敏感性为100%,但Uu对克林霉素的敏感性仅为0%~5.9%。

本实验表明,宫颈炎患者支原体检出率较高,以Uu为主,但常合并Mh感染。因此在临床考虑宫颈炎与支原体相关时,应使用四环素类抗生素——美满霉素和多西环素。如患者不耐受或者需考虑其他病原体如细菌、衣原体等合并感染的可能性时,应进行支原体培养并药敏实验,选择最佳的抗生素或抗生素组合进行治疗,防止支原体反复感染或转成慢性。

参考文献(References)

- [1] 郑华,李明成,丑莉莉.复发性非淋菌性尿道(宫颈)炎支原体的耐药性及相关机制[J].吉林大学学报(医学版),2007,33(5):924-927
Zheng Hua, Bei Ming-cheng, Chou Li-li. Characterization of Mycoplasma isolates and isolates and associated mechanisms of drug resistance in recurring nongonococcal urethritis [J]. Journal of Jilin University(Medicine Edition), 2007, 33(5):924-927
- [2] 其木格,蒋法兴,王千秋,等.解脲脲原体生物群及基因型与黏液脓性宫颈炎相关性的研究[J].中华皮肤科杂志,2010,43(5):312-315
Qi Mu-ge, Jiang Fa-xing, Wang Qian-qiu, et al. Association of Ureaplasma urealyticum biovar and genotype with mucopurulent cervicitis[J]. Chinese Journal of Dermatology, 2010, 43(5):312-315
- [3] Biernat-Sudolska M, Szostek S, Rojek-Zakrzewska D, et al. Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology [J]. Advances in Medical Sciences, 2011, 56(2): 299-303
- [4] 龙辉,汤美芬,崔艳萍.妇科门诊患者宫颈管支原体感染状况及药敏分析[J].现代医院,2012,12(6):78-80
Long Hui, Tang Mei-fen, Cui Yan-ping. The status of cervical canal mycoplasmal infection in obstetric outpatients and its drug sensitivity analysis[J]. Modern Hospital, 2012, 12(6): 78-80
- [5] 杨婧.非淋菌性尿道(宫颈)炎患者泌尿生殖道解脲脲原体及人型支原体的检出率与药敏分析[J].中国皮肤性病杂志,2007,21(12):747-748
Yang Jing. The detection and analysis of drug sensitivity of Ureaplasma Urealyticum and Mycoplasma Hominis [J]. Chinese Journal of Dermatovenereology, 2007, 21(12):747-748
- [6] 沈利蒙,楚旭,董华丽,等.2007-2011年杭州市萧山区女性支原体感染及耐药性分析[J].中国微生态学杂志,2013,25(04):462-465
Shen Li-meng, Chu Xu, Dong Hua-li, et al. Analysis of Mycoplasma infection and drug resistance among female patients with genitourinary tract infection in Hangzhou Xiaoshan district from 2007 to 2011 [J]. Chinese Journal of Microecology, 2013, 25(04): 462-465
- [7] Xie Xin-you, Zhang Jun. Trends in the rates of resistance of Ureaplasma urealyticum to antibiotics and identification of the mutation site in the quinolone resistance-determining region in Chinese patients [J]. FEMS Microbiol Lett, 2006, 259(2):181-186
- [8] 李东辉,颜志中.生殖道解脲脲原体与不孕不育的关系及耐药性分析[J].中华医院感染学杂志,2012,22(3):654-655
Li Dong-hui, Yan Zhi-zhong. Relationship between genital tract-derived ureaplasma urealyticum and infertility and analysis of the drug resistance [J]. Chinese Journal of Nosocomiology, 2012, 22(3): 654-655
- [9] Capoccia, R., G. Greub, D. Baud. Ureaplasma urealyticum, Mycoplasma hominis and adverse pregnancy outcomes[J]. Curr Opin Infect Dis, 2013, 26(3): 231-240

(下转第3486页)

- Parenteral Nutrition: Geriatrics [J]. Clinical Nutrition, 2009, 28(4): 461-466
- [15] Pierre Singer, Mette M. Berger, Greet Van den Berghe, et al. ESPEN Guidelines Parenteral Nutrition: Intensive care [J]. Clinical Nutrition, 2009, 28(4):387-400
- [16] 熊宜军, 邹方友, 周莉. 某院外科 I 类切口预防性应用抗菌药物的合理性分析 [J]. 临床研究, 2012, 10(26):84-85
- Xiong Yi-jun, Zou Fang-you, Zhou Li. Rational analysis of prophylactic antibiotics in Class I surgical incision in a hospital [J]. Clinical Research, 2012, 10(26):84-85
- [17] 吴维玲. 我院 2010 年抗菌药物应用分析 [J]. 海峡药学, 2012, 24(9): 221-222
- Wu Wei-ling. The analysis of antimicrobial drugs used in our hospital in 2010 [J]. Strait Pharmaceutical Journal, 2012, 24(9):221-222
- [18] 曾华勤. 我院抗菌药物的临床应用现状及存在问题分析 [J]. 北方药学, 2012, 9(10):92-93
- Zeng Hua-qin. The analysis of antibacterial drugs in the clinical application in our hospital [J]. Northern Pharmacy, 2012, 9(10):92-93
- [19] Igor N. Sergeev. Genistein induces Ca^{2+} -mediated, calpain/caspase-12-dependent apoptosis in breast cancer cells [J]. Biochemical and Biophysical Research Communications, 2004, 321(2): 462-467
- [20] 蔡威, 王莹. 小儿临床营养管理及其发展现状 [J]. 临床外科杂志, 2012, 20(12):848-850
- Cai Wei, Wang Ying. Pediatric clinical nutrition management and development status [J]. Journal of Clinical Surgery, 2012, 20(12): 848-850

(上接第 3475 页)

- [10] Waites KB, Katz B, Schelonka RL. Mycoplasmas and ureaplasmas as neonatal pathogens [J]. Clin Microbio Rev, 2005, 18(4):757-789
- [11] 韩日新, 杨卫红. 泌尿生殖道解脲支原体感染及药敏试验结果分析 [J]. 检验医学与临床, 2010, 7(07): 672
- Han Ri-xin, Yang Wei-hong. The status of urogenital tract ureaplasma urealyticum infection and drug sensitivity analysis [J]. Laboratory Medicine and Clinic, 2010, 7(07): 672
- [12] 刘朝晖, 张淑增, 任翊. 解脲支原体在正常人群宫颈的存在情况及分群分型 [J]. 中国实用妇科与产科杂志, 2003, 19(03): 161-163
- Liu Zhao-hui, Zhang Shu-zeng, Ren Yi. The existence of ureaplasma urealyticum in cervix in gynecologic check-up population and its biovars and serotypes [J]. Chinese Journal of Practical Gynecology and Obstetrics, 2003, 19(03): 161-163
- [13] 艾静, 王蓓, 于红, 等. 不同女性人群支原体感染状况分析 [J]. 中国公共卫生, 2007, 23(1): 14-15
- Ai Jing, Wang Bei, Yu Hong, et al. Analysis on mycoplasma infection status between healthy women and women with genital tract inflammation [J]. Chinese Journal of Public Health, 2007, 23(1): 14-15
- [14] Waites KB, Crouse DT, Cassell GH. Therapeutic considerations for Ureaplasma urealyticum infections in neonates. Clin Infect Dis, 1993, 17(Suppl 1):S208-S214
- [15] 罗芳. 武汉地区生殖道支原体对四环素类药物的耐药性分析 [J]. 中外医学研究, 2013, 11(6): 69-70
- Luo Fang. Drug resistance analysis to tetracyclines of genital tract mycoplasma in Wuhan area [J]. Chinese and Foreign Medical Research, 2013, 11(6): 69-70
- [16] Xiao L, Crabb DM, Duffy LB, et al. Mutations in ribosomal proteins and ribosomal RNA confer macrolide resistance in human Ureaplasma spp [J]. Int J Antimicrob Ag, 2011, 37(4): 377-379
- [17] Farkas B, Ostorházi E, Pónyai K, et al. Frequency and antibiotics resistance of Ureaplasma urealyticum and mycoplasma hominis in genital samples of sexually active individuals [J]. Orvosi Hetilap, 2011, 152(42):1698-1702
- [18] 张吕良. 900 例宫颈炎患者支原体培养和药敏结果分析 [J]. 广西医科大学学报, 2010, 27(5):722-723
- Zhang Lv-liang. Analysis of detection and susceptibility results for 900 mycoplasma strains from cervicitis [J]. Journal of Guangxi Medical University, 2010, 27(5):722-723
- [19] 王蓓. 支原体在性传播感染中的作用及其耐药机理研究 [D]. 南京: 东南大学, 2007: 43-52
- Wang Bei. Study on the effect and antibiotics resistance mechanism of mycoplasma in sexually transmitted infections [D]. Nanjing: Southeast University, 2007: 43-52
- [20] Xiao L, Crabb DM, Duffy LB, et al. Chromosomal mutations responsible for fluoroquinolone resistance in Ureaplasma species in the United States [J]. Antimicrob Agents Chemother, 2012, 56(5): 2780-2783