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BRIEF COMMUNICATION

EVALUATION OF THE *in vitro* ACTIVITY OF SIX ANTIMICROBIAL AGENTS AGAINST *Neisseria gonorrhoeae*

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SUMMARY

Use of antimicrobials for the treatment of gonorrhea started in 1930 with the utilization of sulfonamides. With the years other drugs were used for its treatment such as penicillin, tetracycline, spectinomycin, and others. Although highly specific in the beginning, these drugs, with time did not show anymore the expected therapeutic results because of aspects of chromosomal and plasmid-mediated resistance. The purpose of this study was to evaluate the susceptibility of *Neisseria gonorrhoeae* strains to six drugs used for its treatment (penicillin, tetracycline, cefoxitin, thiamphenicol, spectinomycin and ofloxacin) by the determination of minimal inhibitory concentrations of these drugs. We concluded that drugs, such as cefoxitin, thiamphenicol and spectinomycin still are excellent pharmacological agents for the treatment of gonorrhea. Penicillin, although still efficient, needs more attention regarding its use, as well as ofloxacin, because of the emergence of resistant strains. Tetracycline and its derivatives should be strongly contraindicated for the treatment of gonorrhea.

KEYWORDS: Gonorrhea; *Neisseria gonorrhoeae*; Drug resistance; Minimum inhibitory concentration.

Gonorrhea is one of the oldest morbid states of humanity. Urethral secretions of venereal origin, supposedly gonococcal, were already reported by the Chinese during Huang Ti's empire in 2,637 BC. The use of antimicrobials in its therapy was started in the 1930s with sulphonamides[7]. However, in spite of being highly efficient, resistance to this drug developed rapidly[4,11], since resistance mechanisms are favored by the selective pressure of its massive use[15]. With the appearance of penicillin, gonorrhea therapy takes a new course. Although in 1943 this drug showed to be highly efficient[16], already by the end of the decade of the 1950s several reports pointed to a reduced susceptibility of the gonococcus to penicillin[8,29]. Since the beginning of the 1980s, reports of resistance to other drugs, such as spectinomycin[25,26] and the cephalosporins[28] also started to emerge. Thus, a good strategy to fight against and control of a certain microbial morbidity should foresee an epidemiological survey program, periodically grading susceptibility behavior of the etiologic agents, among other procedures. The aim of this study was to evaluate the present susceptibility through minimal inhibitory concentration (MIC)[13] determination of the six main drugs used for gonorrhea treatment (penicillin, tetracycline, spectinomycin, cefoxitin, thiamphenicol and ofloxacin).

MATERIAL AND METHODS

This study was performed with *Neisseria gonorrhoeae* strains obtained from patients with acute non-complicated gonorrhea, of both genders, attended by the Sexually Transmitted Diseases Service of the School of Public Health, São Paulo University. In the period from November 2004 to July 2005, 65 strains of *Neisseria gonorrhoeae* were isolated. The strains were isolated in modified Thayer-Martin medium and later identified by direct bacterioscopy with Gram staining; reaction with cytochrome-oxidase and sugar acidification reaction[3,23]. They were also submitted to the chromogenic cephalosporin test for analysis of beta-lactamase enzyme[17]. In the studied population 15 strains were isolated of *Neisseria gonorrhoeae* producing penicillinase (NGPP). All strains were submitted to the susceptibility test by the minimal inhibitory concentration (MIC) test using the method of dilution in Agar[18]. The tested drugs were prepared according to the appropriate techniques[1,10], and in a way to obtain the following final concentrations (in µg/mL): to penicillin, cefoxitin and ofloxacin: 0.125; 0.25; 0.5; 1; 2; 4 and 8 ; to thiamphenicol and tetracycline : 0.125; 0.25; 0.5; 1; 2; 4; 8 and 16 ; to spectinomycin: 7.5; 10; 12.5; 15; 17.5; 20; 32; 64 and 128.

The inocula were prepared from subcultures of each *Neisseria gonorrhoeae*, with a 18-24 hours variation in growth in medium under appropriate conditions[23,24]. The MIC was established by reading the lowest concentration required for total inhibition of bacterial growth. Interpretation of the results, summarized in Table 1, was made according

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to the indications of the National Committee for Clinical Laboratory Standards (NCCLS)[19] (Table 1).

Table 1
Interpretation of the results according NCCLS guideline (µg/mL)

Drug tested	Sensitive	Intermediate	Resistant
Penicillin	≤ 0.06	-	≥ 2
Cefoxitime	≤ 2	4	≥ 8
Thiamphenicol	≤ 2	4	≥ 8
Tetracycline	≤ 0.25		≥ 2
Spectinomycin	≤ 32	64	≥ 128
Ofloxacyn	≤ 0.5	-	≥ 1

RESULTS

The susceptibility test to penicillin revealed that the 50 non-PPNG strains presented an intermediate susceptibility and the 15 PPNG strains showed to be resistant to the drug. Regarding cefoxitin, of the non-PPNG strains, 49 (98%) were susceptible and one (2%) presented intermediate susceptibility, while 93.3% of the PPNG strains were susceptible to this drug.

Regarding thiamphenicol, 76% of the non-PPNG strains showed to be susceptible and 24% presented intermediate susceptibility; of PPNG strains only 59.9% showed to be susceptible.

As concerns susceptibility to tetracycline, 10% of the non-PPNG strains showed to be sensitive, 32% with intermediate susceptibility and 26% resistant, while all PPNG strains were resistant.

Regarding susceptibility to spectinomycin, among the non-PPNG strains 92% showed to be susceptible and 8% had an intermediate susceptibility and, among the PPNG strains 86.4% showed to be susceptible and 13.3% had an intermediate susceptibility.

As regards ofloxacin, 9% of the non-PPNG strains showed to be resistant, while all PPNG strains were susceptible to this drug (Tables 2 and 3).

DISCUSSION

Despite a sharp decline in the incidence of gonococcal infection in developed countries during the last decade, gonorrhea remains one of the most common sexually transmitted infections in developing countries and is a global health problem[12]. Gonococcal resistance to antimicrobial agents is an increasing problem in the treatment of gonorrhea. A high prevalence of plasmid-mediated high-level or a chromosomaly mediated low-level resistance to penicillin or tetracycline has been recognized in many countries[5,14]. By this study, the results obtained regarding cefoxitin showed that non-PPNG strains presented their MIC with higher frequency at 0.125 µg/mL, while PPNG strains showed a higher tolerance towards this drug, presenting a MIC of 0.5 µg/mL with a higher frequency, followed by 2 µg/mL. Among the non-PPNG strains, the mean was 0.515 µg/mL, with the PPNG presenting a mean of 1.616 µg/mL. Thus, a process of tolerance among the *Neisseria gonorrhoeae* strains is of concern. These data are similar to those obtained by DILLON *et al.*[9] and SHIGEMURA *et al.*[22], who identified, respectively, 100% and 90.8% susceptibility to this drug. Regarding susceptibility behavior of thiamphenicol, the two studied populations (non-PPNG and PPNG) presented a homogenous variation. The mean of 1.625 µg/mL observed among the non-PPNG strains is represented by 2.384 µg/mL among the PPNG strains.

Concerning spectinomycin, the variation observed among PPNG strains was higher than that observed among the non-PPNG. We identified 20% of the non-PPNG strains and 13.3% of the PPNG strains to be resistant to this drug, results that are a little higher than those found by DILLON *et al.*[9], RAHMAN *et al.*[21] and SHIGEMURA *et al.*[22].

Regarding tetracycline, our results indicate a high percentage of resistance in both studied populations, with results similar to those of the literature[6,9,22].

Table 2
Distribuition of the 50 non NGPP strains according to MIC of the drugs tested

Drug		0.125	0.25	0.5	1	2	4	12.5	17.5	25.5	32	64	128
Penicillin	N	19	18	12	1								
	%	38	36	24	2								
Cefoxitin	N	34		1	11	3	1						
	%	68		2	22	6	2						
Thiamphenicol	N		3	15	15	5	12						
	%		6	30	30	10	24						
Tetracycline	N		5	24	8	12	1						
	%		10	48	16	24	2						
Spectinomycin	N							1	2	37	6	4	
	%							2	4	74	12	8	
Ofloxacyn	N	2	37	7		1	3						
	%	4	74	14		2	6						

Table 3
Distribution of the 15 NGPP strains according MIC (μg/mL) of the drugs tested

Drug		0.125	0.25	0.5	1	2	4	7.5	8	12.5	15	17.5	20	64
Penicillin	N								15					
	%								100					
Cefoxitin	N		1	6	3	4	1							
	%		6.6	40	20	26.7	6.6							
Thianphen	N		1	3		5	4							
	%		6.6	20		33.3	40							
Tetracycline	N				8	7								
	%				53.3	46.7								
Spectinom	N							4		3	1	1	4	2
	%							26.6		20	6.6	6.6	26.6	13.3
Ofloxacyn	N		10	5										
	%		66.6	33.3										

Table 4
Distribution of the 65 strains of *Neisseria gonorrhoeae* according sensitivity of the drugs tested

	Non PPNG				PPNG			
	Sensitive		Resistant		Sensitive		Resistant	
	N	%	N	%	N	%	N	%
Penicillin	50	100					15	100
Cefoxitin	50	100			15	100		
Thiamph	50	100			15	100		
Tetracycline	37	74	13	26	8	53.3	7	46.7
Spectinom	50	100			15	100		
Ofloxacyn	46	92	4	8	15	100		

As far as penicillin is concerned, the non-PPNG strains presented an intermediated susceptibility, with a tendency to increase in susceptibility to this drug with time, although the obtained results are inferior to those found by CHOWDRY *et al.*[6] and SHIGEMURA *et al.*[22]. Regarding ofloxacin, we identified only 8% resistance among the non-PPNG strains, a much inferior result than those reported in the world literature, which oscillate between 70% and 90% of resistance[2,20,27], these probably being the first resistant strains identified in Brazil.

The detection of strains other than NGPPs that were presented with MIC and that fell in the intermediate classification of sensitivity between cefoxitime and thiamphenicol, and of NGPP strains with intermediate MIC of sensitivity between thiamphenicol and spectinomycin, and strains with MIC above the sensitivity limits to ofloxacyn, is a warning signal. It reinforces the need for the use of more rigorous criteria in the prescription of these drugs that, in spite of still being highly effective in the treatment of gonorrhea, already present indications in the laboratory that suggest they may also be heading toward a loss of effectiveness in the treatment of the disease, with the possibility of allowing the emergence of chromosomal or even plasmidial resistance to these drugs.

CONCLUSION

In conclusion, drugs like cefoxitin, thiamphenicol, spectinomycin and ofloxacin constitute, even today, excellent drugs for the treatment of gonorrhea. Penicillin, in spite of being still efficient, requires more attention as to its use in view of emergence of resistant strains. Regarding tetracycline, its use at present is daring and its prescription should be absolutely contraindicated. In view of the obtained results it can be concluded that most of the tested drugs presented satisfactory results in the treatment of gonorrhea.

RESUMO

Avaliação *in vitro* da atividade de seis drogas antimicrobianas contra *Neisseria gonorrhoeae*

A utilização de antimicrobianos no tratamento da gonorréia iniciou-se em 1930 com a utilização das sulfonamidas. No decorrer dos anos outras drogas passaram a ser utilizadas em seu tratamento como a penicilina, tetraciclina, espectinomicina e outras. Embora altamente eficazes no início, essas drogas, ao longo do tempo, passaram a não mais apresentar o resultado terapêutico esperado em virtude do aparecimento de quadros de resistência cromossômica e plasmidial. Este trabalho teve por objetivo avaliar a sensibilidade de cepas de *Neisseria gonorrhoeae* a seis drogas utilizadas no seu tratamento (penicilina, tetraciclina, cefoxitina, tianfenicol, espectinomicina e ofloxacina) através da concentração inibitória mínima. Concluimos que drogas como a cefoxitina, o tianfenicol e a espectinomicina ainda constituem excelentes fármacos para o tratamento da gonorréia. A penicilina, embora ainda eficaz, enseja maiores cuidados na sua utilização, assim como a ofloxacina, frente ao surgimento de cepas resistentes e, a tetraciclina e seus derivados deve ser sobremaneira contra-indicada no tratamento da gonorréia.

REFERENCES

1. ANHALT, J.P. & WASHINGTON II, J.A. - Preparation and storage of antimicrobial solutions. In: LENNETTE, E.H.; BALLOWS, A. & HAUSLER, W.J. **Manual of clinical microbiology.** 4. ed. Washington, American Society for Microbiology, 1985. p. 1019-1020.

2. BAUER, H.M.; MARK, K.E.; SAMUEL, M. *et al*. - Prevalence of and associated risk factors for fluoroquinolone-resistant *Neisseria gonorrhoeae* in California, 2000-2003. **Clin. infect. Dis., 41:** 795-803, 2005.

3. CARLSON, B.L.; CALNAN, M.B. & GOODMAN, R.E. - Phadebact monoclonal GC-OMMI test for confirmation of *Neisseria gonorrhoeae*. **J. clin. Microbiol., 25:** 1982-1984, 1987.

4. CARPENTER, C.M.; ACKERMAN, H. & WINCHESTER, M.E. - Correlation of *in vitro* sulfonamide resistance of the gonococcus with results of sulfonamide therapy. **Amer. J. publ. Hlth., 34:** 250-254, 1944.

5. CHALKLEY, L.J.; JANSE van RENSBURG, M.N.; MATTHEE, P.C.; ISON, C.A. & BOTHA, P.L. - Plasmid analysis of *Neisseria gonorrhoeae* isolates and dissemination of TeT M genes in southern Africa 1993-1995. **J. Antimicrob. Chemother**., **40:** 817-822, 1997.

6. CHOWDHRY, S.; PANDHI, D.; VIDHANI, S.; BHALLA, P. & REDDY, B.S. - High incidence of treatment failure of *Neisseria gonorrhoeae* isolates to ciprofloxacin in male gonococcal urethritis in Delhi. **Int. J. STD AIDS., 13:** 564-567, 2002.

7. COOKKINIS, A.J. & McELLIGOT, G.L.M. - Sulphanilamide in gonorrhea. **Lancet**, 2: 355-362, 1938.

8. CURTIS, F.R. & WILKINSON, A.E. - A comparison of the *in vitro* sensitivity of gonococci to penicillin with the results of treatment. **Brit. J. vener. Dis., 34:** 70-78, 1958.

9. DILLON, J.A.; LI, H.; SEALY, J.; RUBEN, M. *et al.* - Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates from three Caribbean countries: Trinidad, Guyana and St. Vincent. **Sex. transm. Dis., 28:** 508-514, 2001.

10. FINEGOLD, S.M. & BARON, E.J. - Metodos para evaluar la efectividad antimicrobiana. In: **BAILEY & SCOTT diagnóstico microbiologico.** 7. ed. Buenos Aires, Medica Panamericana, 1989. p. 190-210.

11. GOODALE, W.T.; GOULD, R.G. & SCHWALB, L. - Laboratory identification of sulfonamide-resistant gonococci infections**. J. Amer. med. Ass., 123:** 547-549, 1943.

12. ISON, C.A.; DILLON, J.A. & TAPSALL, J.W. - The epidemiology of global antibiotic resistance among *Neisseria gonorrhoeae* and *Haemophilus ducreyi*. **Lancet**, **351** (suppl. 3): 8-11, 1998.

13. KNAPP, J.S. - Laboratory methods for the detection and phenotypic characterization of *Neisseria gonorrhoeae* strains resistant to antimicrobial agents. **Sex. transm. Dis**., **15:** 225-231, 1988.

14. KNAPP, J.S.; MESOLA, V.P.; NEAL, S.W. *et al*. - Molecular epidemiology in 1994, of *Neisseria gonorrhoeae* in Manila and Cebu city, Republic of the Philippines. **Sex**. **transm. Dis**., **24:** 2-7, 1997.

15. LANKFORD, C.E. - The *in vitro* tolerance of the gonococcus for penicillin. **Amer. J**. **Syph**., **29:** 56-63, 1945.

16. MAHONEY, J.F.; FERGUSON, C. & BUCHHOLTZ, M. - The use of penicillin sodium in the treatment of sulfonamide-resistant gonorrhea in men: a preliminary report. **Amer. J. Syph., 27:** 525-528, 1943.

17. MONTGOMERY, K.; RAYMOND, L. & DREW, W.L. - Chromogenic cephalosporin spot test to detect beta-lactamase in clinically significant bacteria. **J. clin. microbiol., 9:** 205-207, 1979.

18. NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS - Methods for dilution antimicrobial susceptibility for bacteria that grow aerobically. Villa Nova, NCCLS, 1985. (Approved standard. M7-A5).

19. NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS - Performance standards antimicrobial susceptibility testing: second informational supplement. Villa Nova, NCCLS, 1987. (M-100,52)

20. NG, L.K.; SAWATZKY, P.; MARTIN, I.E. & BOOTH, S. - Characterization of ciprofloxacin resistance in *Neisseria gonorrhoeae* isolates in Canada. **Sex. transm. Dis., 29:** 780-788, 2002.

21. RAHMAN, M.; SULTAN, Z.; MONIRA, S. *et al*. - Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolated in Bangladesh (1997 to 1999): rapid shift to fluoroquinolone resistance. **J. clin. Microbiol., 40:** 2037-2040, 2002.

22. SHIGEMURA, K.; OKADA, H.; SHIRAKAWA, T. *et al*. - Susceptibilities of *Neisseria gonorrhoeae* to fluoroquinolones and other antimicrobial agents in Hyogo and Osaka, Japan. **Sex. transm. Infect., 80:** 105-107, 2004.

23. SIQUEIRA, L.F.G. - O laboratório nas doenças sexualmente transmissíveis III**. Bol. Inform. Union, 34:** 3-4, 1984.

24. STIERS, E.; FOLTZ, E.L. & GRAVES, B. - A inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. **Antimicrob. Agents. Chemother., 9:** 307-311, 1959.

25. STOLZ, E.; ZWART, H.G.F. & MICHEL, M.F. - Activity of eight antimicrobial agents *in vitro* against *Neisseria gonorrheae*. **Brit. J. vener. Dis., 51:** 257-264, 1975.

26. THORNSBERRY, C.; JAFFE, H. & BROWN, S.T. - Spectinomycin resistant *Neisseria gonorrheae*. **J. Amer. med. Ass., 237:** 2405-2406, 1977.

27. UTHMAN, A.; HELLER-VITOUCH, C.; STARY, A. *et al*. - High-frequency of quinolone-resistant *Neisseria gonorrhoeae* in Austria with a common pattern of triple mutations in GyrA and ParC genes. **Sex. transm. Dis., 31:** 616-618, 2004.

28. ZENILMAN, J.M.; NIMS, L.J.; MENEGUS, M.A.; NOLTE, F. & KNAPP, J.S. - Spectinomycin resistant gonococcal infections in the United States 1985-1986. **J. infect. Dis., 156:** 1002-1004, 1987.

29. WILLCOX, R.R. - Treatment problems of gonorrhea**. Bull. Wld Hlth Org., 24:** 307-319, 1961.

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