J Infect Chemother 22 (2016) 194–197

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Note

In vitro potency and combination testing of antimicrobial agents against *Neisseria gonorrhoeae*

Amrita Bharat [a], Irene Martin [a], George G. Zhanel [b], Michael R. Mulvey [a, *]

[a] *Bacteriology and Enteric Diseases Program, National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St, Winnipeg, Manitoba, R3E 3R2, Canada*
[b] *Department of Medical Microbiology and Infectious Diseases, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, R3A 1R9, Canada*

ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form
6 October 2015
Accepted 9 October 2015
Available online 18 November 2015

Keywords:
Neisseria gonorrhoeae
Antimicrobial susceptibility
Antimicrobial combinations
Synergy

ABSTRACT

Antimicrobial resistant *Neisseria gonorrhoeae* is a major concern to public health due to decreased susceptibility to frontline antimicrobials. To find agents that are active against *N. gonorrhoeae*, we tested antimicrobials alone or in combination by Etest gradient strips. The potencies (as assessed by minimum inhibitory concentrations) of twenty-five antimicrobials were evaluated against nine reference strains of *N. gonorrhoeae* (WHO F, G, K, L, M, N, O, P and ATCC 49226). Potency was greatest for netilmicin, quinupristin-dalfopristin, ceftriaxone, ertapenem and piperacillin-tazobactam. Combinations of azithromycin, moxifloxacin, or gentamicin with ceftriaxone, doripenem, or aztreonam were tested against reference isolates and the fractional inhibitory concentration index (FICI) was calculated. All nine combinations resulted in indifference (>0.5 FICI $\leq$ 4). Combinations with FICI < 1 were further evaluated in nine clinical isolates which supported the finding of indifference. No antagonism was observed in any of the combinations tested. This is the first report in which the six combinations of azithromycin, moxifloxacin or gentamcin in combination with doripenem or aztreonam were tested in *N. gonorrhoeae*. These data on antimicrobials with higher potency and combinations that did not show antagonism can help to guide larger scale susceptibility studies for antimicrobial resistant *N. gonorrhoeae*.

Gonorrhea is the second most common bacterial sexually transmitted infection after chlamydia, with an estimated 106 million new infections each year globally [1]. The World Health Organization and the US Centers for Disease Control and Prevention have named antimicrobial resistant gonococcus as an important concern to human health [2]. Previously, gonorrhea infections were treated with sulfonamides, penicillin, tetracycline, spectinomycin, ciprofloxacin or erythromycin, however each drug had to be discontinued due to resistance [2]. The current recommended treatment in Europe, the United States and Canada is combinational therapy with azithromycin and ceftriaxone or alternatively, cefixime, however, decreased susceptibility and treatment failures with these last remaining recommended treatments have been reported around the world [2], prompting efforts to explore alternative treatment options.

In addition to new compounds in the antimicrobial development pipeline, potential treatments may include discontinued antimicrobials, which may regain efficacy if the resistance mechanisms are lost over time. Combinations of antimicrobials may act synergistically, where the effect of two antimicrobials in combination is greater than the sum of the effects of each antimicrobial acting alone [3]. Further, combinations of antimicrobials can have similar advantages as cotherapy with azithromycin and ceftriaxone, including increased efficacy against: (i) mono-resistant isolates; (ii) pharyngeal infections; and (iii) co-infections with *Chlamydia trachomatis* as long as the antibiotics are not antagonistic in combination. The only report of antimicrobial synergy in *N. gonorrhoeae* was between azithromycin and cefixime in Japanese isolates [4]; however, synergy with these drugs was not observed in later studies [5,6]. All other antimicrobial combinations that were tested in *N. gonorrhoeae* have not produced synergy [4–9].

To look for antimicrobials that are active against *N. gonorrhoeae*, we tested the potency of 25 antimicrobial agents, representing 12 structural and mechanistic classes, including inhibitors of cell wall synthesis, protein synthesis, DNA synthesis, RNA synthesis, and

* Corresponding author. Tel.: +1 (204) 789 2133; fax: +1 (204) 789 5020.
E-mail address: michael.mulvey@phac-aspc.gc.ca (M.R. Mulvey).

http://dx.doi.org/10.1016/j.jiac.2015.10.002
1341-321X/

metabolism. This antimicrobial panel included older antimicrobials, newer derivatives and drugs whose activities against gonococcus have not been reported in the literature. We also tested nine combinations of antimicrobials to look for potential enhancement of activity when antimicrobials are used together.

Twenty-five antimicrobials were tested against nine reference strains of *N. gonorrhoeae* (Table 1). The WHO isolates (WHO F, G, K, L, M, N, O and P) are a panel of reference strains that provide global quality assurance for surveillance of antimicrobial resistance [10], while ATCC 49226 is the reference strain recommended by the Clinical Laboratory Standards Institute for *N. gonorrhoeae* susceptibility testing [11]. These WHO reference strains represent a range of genotypes at all of the known loci of antimicrobial resistance (*penA*, *mtrR*, *porB*, *ponA*, *gyrA* and *parC*) as described by Unemo et al. [10]. Susceptibilities were determined by antimicrobial gradient epsilometer test (Etest) according to the manufacturer's protocol (bioMérieux, St Laurent, QC, Canada) with growth on GC medium base plus Kelloggs supplements at 35 °C in a 5% CO_2 atmosphere for 20 h. The Etest minimum inhibitory concentration (MIC) were tested in triplicate and the average value was taken.

For quality control, we also performed agar dilution MICs according to CLSI guidelines [11] for six antimicrobials: tetracycline, spectinomycin, cefixime, ceftriaxone, penicillin, and ciprofloxacin. The Etest MICs for ATCC 49226 were within the acceptable ranges that are specified by CLSI for this reference strain. Further, for these six antibiotics against all nine strains, 96% of the Etest MICs were within two doubling dilutions of the agar dilution MICs. This rate of agreement is consistent with the results of a larger scale comparison of Etest and agar dilution [13].

Amongst the aminoglycosides, low MICs were obtained for gentamicin (3 μg/mL–4 μg/mL) and netilmicin (1.7 μg/mL–3.3 μg/mL); however, MICs for amikacin were higher (32 μg/mL–75 μg/mL). Gentamicin is being investigated as a possible treatment for gonorrhea [2]. Relatively low MICs were obtained for the protein synthesis inhibitors, quinupristin-dalfopristin (0.15 μg/mL–1.3 μg/mL), which is normally used for the treatment of Gram-positive infections. Together quinupristin and dalfopristin produce synergistic enhancement of binding to the 50S ribosomal subunit.

Elevation of β-lactam MICs is most strongly associated with variations in penicillin binding protein 2 (PBP2, encoded by *penA*) [12], which has a mosaic sequence in WHO K and an A501V substitution in WHO L. The cephalosporin antimicrobials had the weakest activity against strain WHO K (Table 1). Although the fourth generation cephalosporin, cefepime (1.5 μg/mL), was more active against WHO K than cefuroxime (7.3 μg/mL), it was not more active than ceftriaxone (0.074 μg/mL) and cefixime (0.17 μg/mL) suggesting that this newer cephalosporin will likely not be an improvement over current treatments unless the breakpoint for resistance to cefepime is higher. Other active cell wall synthesis inhibitors included ertapenem (0.004 μg/mL–0.05 μg/mL), which is being investigated as a potential future treatment for gonorrhea infections [2]. Piperacillin is a newer derivative of penicillin that was tested here in combination with the β-lactamase inhibitor, tazobactam. MICs of piperacillin-tazobactam (≤0.016 μg/mL–0.08 μg/mL) were lower than MICs of penicillin (≤0.016–32 μg/mL). For penicillin producing *N. gonorrhoeae* (PPNG) strains (WHO M, WHO N and WHO O), the penicillin MICs were approximately 32 μg/mL while the piperacillin-tazobactam MICs were significantly lower at ≤0.016 μg/mL. For the remaining strains, which were non-PPNG, the piperacillin-tazobactam MICs were on average 17-fold lower than the penicillin MICs suggesting that piperacillin is a more

Table 1
Potency of 25 antimicrobials against nine reference strains of *N. gonorrhoeae*.

	MIC (μg/mL)[a]								
	WHO F	WHO G	WHO K	WHO L	WHO M	WHO N	WHO O	WHO P	ATCC 49226
Amikacin	32	43	37	37	35	75	64	48	53
Gentamicin	2.7	3.2	2.7	2.7	2.8	3.7	3.3	3.0	3.3
Netilmicin	**1.7**	**1.7**	**1.7**	**1.8**	**1.7**	**2.0**	**2.3**	**3.3**	**2.2**
Minocycline	0.10	20	0.75	0.75	0.75	21	0.50	1.17	0.58
Tetracycline	0.15	32	1.83	1.25	1.50	21	1.50	1.00	0.58
Erythromycin	0.07	0.10	0.46	0.42	0.38	0.29	0.42	3.0	0.92
Azithromycin	0.08	0.10	0.19	0.23	0.18	0.13	0.21	3.3	0.50
Spectinomycin	5.33	7.3	8.0	8.0	8.7	8.0	>1024	12	12
Quinupristin-Dalfopristin	**0.15**	**0.17**	**0.38**	**0.38**	**0.38**	**0.46**	**0.42**	**1.33**	**0.63**
Cefuroxime	0.02	0.09	7.33	1.67	0.10	0.15	0.25	0.06	0.38
Cefixime	<0.016	<0.016	0.17	0.04	<0.016	<0.016	<0.016	<0.016	<0.016
Ceftriaxone	**<0.002**	**0.008**	**0.074**	**0.094**	**0.011**	**0.007**	**0.019**	**0.007**	**0.013**
Cefepime	<0.016	0.03	1.5	0.19	0.03	0.03	0.04	0.03	0.06
Ertapenem	**<0.002**	**0.004**	**0.047**	**0.006**	**0.006**	**0.005**	**0.008**	**0.007**	**0.012**
Doripenem	0.01	0.03	0.46	0.02	0.02	0.03	0.04	0.04	0.05
Meropenem	0.005	0.013	0.125	0.013	0.015	0.015	0.024	0.017	0.021
Penicillin	<0.016	0.2	0.9	1.3	20	32	32	0.3	0.8
Piperacillin-Tazobactam	**<0.016**	**<0.016**	**0.05**	**0.08**	**<0.016**	**<0.016**	**0.02**	**<0.016**	**0.02**
Aztreonam	0.03	0.13	4.7	0.38	0.10	0.11	0.23	0.17	0.29
Colistin	106.67	85	>256	117	107	>256	>256	>256	128
Polymyxin B	96.00	85	213	59	80	384	277	427	96
Moxifloxacin	0.003	0.03	5.3	7.3	0.71	0.92	0.01	0.02	0.01
Ciprofloxacin	0.003	0.094	>32	>32	1.25	4.67	0.007	0.004	0.003
Rifampicin	0.09	0.13	0.21	0.15	>32	>32	0.17	>32	0.21
Trimethoprime-Sulfameth-oxazole	0.17	2.0	1.0	0.16	0.79	1.17	0.75	0.75	0.05

Bold text highlights potent antimicrobials that are discussed in the text.
Horizontal lines group antimicrobials with similar mechanism of action.
[a] Performed according to CLSI guidelines for *N. gonorrhoeae*.