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Antimicrobial Susceptibility of Clinical Isolates of *Neisseria gonorrhoeae* to Unconventional Antimicrobials with Therapeutic Potential: Results of the Canadian Antimicrobial Resistance Initiative for *Neisseria gonorrhoeae* (CARING)

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ABSTRACT

Background: Gonococcal infections are increasing in prevalence in many parts of the world. Increasing prevalence is compounded by increasing resistance to first line antimicrobial therapy, particularly the imminent risk of cephalosporin resistance. Drug-resistant *N. gonorrhoeae* (NG) has been declared an urgent threat by the Centers for Disease Control, and prevalence of isolates with reduced susceptibility to cefixime have been observed in 1-2% of American isolates in recent years. There are few novel antimicrobial agents with activity against NG in development, and treatment may become increasingly difficult. The purpose of this study was to evaluate the activity of currently marketed antimicrobial agents against Canadian NG isolates.

Methods: 75 NG isolates were recovered from a variety of clinical sources from across Canada. Antimicrobial susceptibility testing to penicillin, spectinomycin, tetracycline, erythromycin, ceftriaxone, ciprofloxacin, cefixime, azithromycin, gentamicin and ertapenem were performed by agar dilution at the National Microbiology Laboratory and susceptibility testing to netilmicin, fosfomycin, tigecycline, linezolid, ceftaroline and ceftazidime-avibactam were performed as part of the Canadian Antimicrobial Resistance Initiative for *Neisseria gonorrhoeae* (CARING). Where available, susceptibility results were interpreted using CLSI M100-S25 breakpoints.

Results: Presented in Table 1.

Conclusions: Aminoglycosides, ceftazidime-avibactam, ceftaroline, ertapenem, tigecycline, fosfomycin and linezolid are active versus NG. Additional studies are required with these agents and other unconventional agents to determine what role they may have in the treatment of GC either alone or as part of combination therapy.

BACKGROUND

The ideal treatment for gonorrhea is a highly potent antimicrobial with a long half-life that is orally bioavailable; allowing effective empiric single dose therapy at the time of presentation. For years, fluoroquinolones and oral third generation cephalosporins such as cefixime were the choice of therapy because these criteria were met. However, multi-drug resistant *N. gonorrhoeae* is now a growing concern world-wide (1). Emergence of cephalosporin-resistant isolates has occurred in isolated areas and reduced susceptibility to cephalosporins remains uncommon but is widespread (1). Azithromycin resistance is also reported globally and resistance to fluoroquinolones, tetracyclines and penicillin is now common and widespread (1). With isolates with reduced cephalosporin susceptibility increasing in North America (2, 3), a crisis of untreatable gonorrhea is looming (1). The purpose of this study was to investigate the *in vitro* activity of various currently marketed antimicrobials against a collection of *N. gonorrhoeae* isolates from across Canada.

MATERIALS & METHODS

Isolates were submitted to the National Microbiology Laboratory as part of the Public Health Agency of Canada National Surveillance strategy for *N. gonorrhoeae*. Isolates are submitted when provincial laboratories identify resistance to at least one antibiotic or if the provincial laboratories do not perform any antimicrobial susceptibility testing. Between 900 and 1200 isolates are submitted per year (2). A sample of 75 isolates were selected for this study. Minimum inhibitory concentration (MIC) was determined by agar dilution (4) on GC base to the following conventional antimicrobials: penicillin, ceftriaxone, cefixime, ciprofloxacin, spectinomycin, tetracycline, erythromycin, azithromycin, gentamicin, netilmicin, ceftaroline, ertapenem, ceftazidime-avibactam, fosfomycin, tigecycline and linezolid. Where available, CLSI breakpoints were used to interpret MICs (5).

RESULTS

TABLE 1: MIC_{50}, MIC_{90} and susceptibility of isolates to conventional and unconventional antimicrobials

	All isolates (n=75), µg/mL				
	MIC_{min}	MIC_{max}	MIC_{50}	MIC_{90}	% Susceptible
Penicillin	0.06	>256	1	16	5.3%
Ceftriaxone	0.002	0.12	0.015	0.12	100%
Cefixime	0.002	0.5	0.015	0.25	97.3%
Ciprofloxacin	0.002	32	0.015	16	57.3%
Spectinomycin	16	64	16	32	98.7%
Tetracycline	0.25	64	4	32	4%
Erythromycin	0.06	>64	1	8	N/A
Azithromycin	0.03	16	0.25	4	N/A

	All isolates (n=75), µg/mL				
	MIC_{min}	MIC_{max}	MIC_{50}	MIC_{90}	% Susceptible
Gentamicin	2	16	8	16	N/A
Netilmicin	1	8	4	8	N/A
Ceftaroline	≤0.008	0.5	0.06	0.12	N/A
Ertapenem	0.008	0.5	0.06	0.25	N/A
Ceftazidime-avibactam	≤0.06	2	0.12	1	N/A
Fosfomycin	8	64	16	32	N/A
Tigecycline	0.06	1	0.25	0.5	N/A
Linezolid	1	16	4	8	N/A

TABLE 2: MIC_{50}, MIC_{90} and susceptibility of cefixime non-susceptible isolates

	Isolates with reduced cefixime susceptibility[1] (n=10), µg/mL				
	MIC_{min}	MIC_{max}	MIC_{50}	MIC_{90}	% Susceptible
Penicillin	1	4	2	2	0%
Ceftriaxone	0.06	0.12	0.12	0.12	100%
Cefixime	0.25	0.5	0.25	0.5	80%
Ciprofloxacin	8	32	16	16	0%
Spectinomycin	16	32	16	32	100%
Tetracycline	1	4	2	4	0%
Erythromycin	0.5	≥64	1	4	N/A
Azithromycin	0.12	8	0.12	1	N/A

[1]Defined as MIC >0.12 µg/mL

	Isolates with reduced cefixime susceptibility[1] (n=10), µg/mL				
	MIC_{min}	MIC_{max}	MIC_{50}	MIC_{90}	% Susceptible
Gentamicin	4	16	8	8	N/A
Netilmicin	4	8	4	8	N/A
Ceftaroline	0.12	0.5	0.12	0.5	N/A
Ertapenem	0.25	0.5	0.25	0.25	N/A
Ceftazidime-avibactam	0.5	2	1	1	N/A
Fosfomycin	16	32	16	32	N/A
Tigecycline	0.06	1	0.12	0.5	N/A
Linezolid	4	8	4	8	N/A

[1]Defined as MIC >0.12 µg/mL

A high susceptibility rate was observed for cefixime, ceftriaxone and spectinomycin.

Using 2007 FDA breakpoints (6) for azithromycin (MIC ≤ 1 µg/mL), 82.7% of isolates were susceptible to azithromycin. Using EUCAST breakpoints (7) (MIC ≤ 0.25 µg/mL), 57.3% of isolates were susceptible.

Among marketed unconventional antimicrobials, activity was generally very good and except for linezolid, nearly all isolates had MICs below the susceptibility breakpoints of other organisms for which the agents are used.

Gentamicin and netilmicin were approximately 2 and 4 times more potent, respectively, than spectinomycin.

Among isolates with reduced susceptibility to cefixime (MIC ≥ 0.25 µg/mL), ceftriaxone and spectinomycin remained active.

Among isolates with reduced susceptibility to cefixime (MIC ≥ 0.25 µg/mL), activity of the unconventional non-beta-lactam antimicrobials was comparable to the activity among those with low cefixime MIC.

A strong correlation between MICs of all the beta-lactams was observed, supporting a common mechanism of reduced susceptibility to all the beta-lactams.

CONCLUSIONS

Cefixime and ceftriaxone resistance remains uncommon in Canada.

The occurrence of elevated MICs to azithromycin is concerning, but may represent selection bias in both the sample of isolates tested in this study and the reference laboratory submission criteria.

Most of the unconventional antimicrobials tested had good activity against *N. gonorrhoeae* and may be therapeutically useful. MIC_{50} and MIC_{90} values were comparable to other organisms for which these agents are considered therapeutically useful. However, additional studies, including PK/PD experiments and clinical trials are warranted.

Gentamicin and netilmicin were more potent than spectinomycin against this collection of *N. gonorrhoeae* and could be therapeutically useful provided phamacokinetics are similar. Gentamicin is used as treatment for gonococcal infection in some countries providing further evidence of therapeutic utility.

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