ACTIVITY OF FAROPENEM AGAINST *NEISSERIA GONORRHOEAE* INCLUDING CONTEMPORARY ANTIMICROBIAL RESISTANCE PHENOTYPES

R.N. JONES, I.A. CRITCHLEY, N. JANJIC, S. POTTUMARTHY

JMI Laboratories, North Liberty, IA; Replidyne Inc., Louisville, CO

E-312

ABSTRACT

Background: There is a worldwide concern about the escalating resistance (R) among *N. gonorrhoeae* (NG) to first line antimicrobials especially R to single-dose fluoroquinolones (QRNG) in the Far East. There is an urgent need for potent agents with high therapeutic efficacy (> 95.0% cure). Faropenem (FAR), a penem with high potency and wide-spectrum of activity, was evaluated against NG isolates pre-selected for prevailing R phenotypes.

Methods: 265 NG strains were analyzed: 189 recent genital tract strains (2004) from USA states where QRNG has become prevalent; 24 bacteremic strains and 76 well-characterized R phenotypes (53 QRNG [31 from Japan, 1990s], 23 with reduced S to penicillin [PEN] and/or tetracycline [TET]). Agar dilution MICs were determined using GC agar base supplemented with IsoVitaleX or defined CLSI supplement. FAR activity was compared to: PEN, TET, ceftriaxone (CTX), cefuroxime (XM), azithromycin (AZT), ciprofloxacin (CIP) and levofloxacin (LEV).

Results: FAR activity was adversely influenced by L-cysteine in IsoVitaleX (4X MIC_{90} increase; 0.06 vs 0.25 µg/ml). R among NG to CIP, PEN and TET was ≥ 24.2%. Rank order of potency was: CTX (MIC_{90}, 0.06 µg/ml) > FAR (0.25) > AZT (0.5) > XM (1) > TET (2) > PEN = CIP = LEV (4). FAR was 4X more active than oral XM (MIC_{90}, 0.25 vs 1 µg/ml) but 4X less active than CTX (0.25 vs 0.06 µg/ml). Activity of FAR was not affected by penicillinases (PPNG, MIC_{90} 0.12 µg/ml), but non-PPNG-PEN-R resulted in 16X increase in MIC_{90} (0.016 PEN-S to 0.25 µg/ml PEN-R). While the QRNG rates for 6 states ranged from 0.0% (Ohio) to 66.7% (Hawaii), FAR retained potency (MIC_{90}, ≤ 0.25 mg/ml) against these strains and historic QRNG.

Conclusions: FAR activity should be determined on L-cysteine-free agar by CLSI methods. Ability of FAR to maintain acceptable potency irrespective of the R phenotype makes it a promising candidate for therapeutic development as an oral-ß-lactam for uncomplicated NG infections where QRNG has become prevalent.

INTRODUCTION

- Although humans are the only known reservoir for *Neisseria gonorrhoeae*, the control and eradication of this sexually transmitted disease have remained elusive over decades. While the frequency of asymptomatic infection, lack of innate or acquired immunity and changes in human sexual behavior have all contributed to the continued spread of gonococcal infections, a principal contributing factor has been the remarkable ability of *N. gonorrhoeae* to acquire antimicrobial resistances. Renewed concerns in the treatment and control of gonococcal infections in recent years has been spurred by: 1) reports of increasing incidence of gonococcal infections in various geographical locations worldwide; 2) escalating incidence of fluoroquinolone resistance, reaching > 95.0% in certain locales; and 3) inconsistent availability of the potent orally administered cefixime, commonly recommended as the agent of "first choice". In the United States (USA), gonorrhoea has become the second most frequently reported communicable disease with a rate of 116.2 per 100,000 persons (335,104 cases reported in 2003), which was 6-fold higher than the target to be achieved in 5 years by the "Healthy People 2010 objective" of 19.0 cases per 100,000.
- *N. gonorrhoeae*, is a prime example of a 'panmictic' organism with a striking capacity for genetic recombination, and acquisition of resistance to the recommended therapeutic agents. The introduction of fluoroquinolones as single-dose, orally administered agents was soon followed by the spread of fluoroquinolone-resistant *N. gonorrhoeae* (QRNG) in the 1990's. In the USA, QRNG were first reported in Hawaii (1991), and now constitute ≥ 20.0% of the gonococcal isolates in that region, with a 4.2% overall QRNG rate from the 30 participating cities in the Gonococcal Isolate Surveillance Project (GISP). In the face of this emerging resistance threat, the anti-gonococcal efficacy of a number of newer fluoroquinolones with dual action targeting topoisomerases (DNA gyrase and topoisomerase IV) such as garenoxacin, gatifloxacin, gemifloxacin, sitafloxacin or trovafloxacin have been studied *in vitro*; however, their clinical efficacy has not been widely investigated.
- Consequently, there is an urgent need for safe, alternative anti-gonococcal compounds that can be administered orally and have effective potency allowing high therapeutic efficacy (> 95.0% cure rate) with preferably a single-dose regimen. We evaluated the anti-gonococcal activity of faropenem (a ß-lactam penem antimicrobial) along with seven comparator, reference antimicrobials against a collection of 265 gonococcal isolates.

PATIENTS AND METHODS

STRAIN COLLECTION

A collection of clinical isolates of *N. gonorrhoeae*, inclusive of 189 (71.3%) contemporary strains isolated from clinical specimens in 2004 enriched with 76 (28.7%) well-characterized resistant phenotypes of *N. gonorrhoeae* from the Jones Microbiology Institute (JMI; North Liberty, Iowa) laboratories collection were analyzed. The 189 recent clinical strains were obtained from six geographically distinct areas where QRNG has been reported to be endemic/epidemic and include: California (7; 3.7%), Hawaii (27; 14.3%), New York (25; 13.2%), Ohio (36; 19.1%), Oregon (18; 9.5%), and Washington State (76; 40.2%). The 76 well-characterized resistant phenotypes included: 53 QRNG isolates; 15 strains resistant to penicillin and tetracycline; and eight isolates with intermediate susceptibility to penicillin by mechanisms analysis. The majority of the QRNG isolates (51 of 53; 96.2%) were isolated in the Far East and characterized in Japan (courtesy of Professors Tanaka and Deguchi). More than half (31; 60.8%) of these isolates have documented mutations in the quinolone-resistance-determining-regions (QRDR) with single aminoacid substitutions in GyrA, 20 strains (Ser-91→Phe, 14 strains; Asp-95→Asn, 4 strains; and Ser-91→Tyr, 2 strains); single aminoacid substitutions in both GyrA and ParC, 8 strains (GyrA [Ser-91→Phe], ParC [Asp-86→Asn], 6 strains) and (GyrA [Ser-91→Phe], ParC [Ser-87→Ile], 2 strains); and double aminoacid substitutions in GyrA and single substitution in ParC, 3 strains (GyrA [Ser-91→Phe, Asp-95→Asn], ParC [Ser-88→Pro], 2 strains) and (GyrA [Ser-91→Phe, Asp-95→Gly], ParC [Glu-91→Gly], 1 strain).

SUSCEPTIBILITY TESTING METHODS

MICs of eight antimicrobial agents were determined by the reference agar dilution method using GC agar base supplemented with 1% IsoVitaleX with L-cysteine (BBL, Becton Dickinson) or CLSI (2005) defined supplement which is most similar to IsovitaleX but without the 25.9 g of L-cysteine hydrochloride component. The antimicrobial agents tested were faropenem (from Replidyne, Inc., Louisville, CO), azithromycin, ceftriaxone, cefuroxime, ciprofloxacin, levofloxacin, penicillin and tetracycline, and the results were interpreted according to the CLSI M100-S15 standard. For levofloxacin, the breakpoint criteria for ofloxacin were used, corrected for the 2-fold greater potency for levofloxacin; susceptible at ≤ 0.12 mg/ml; resistant at ≥ 1µg/ml. The results were validated using the following quality control strains: *N. gonorrhoeae* ATCC 49226 and *Staphylococcus aureus* ATCC 29213.

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RESULTS

- For the entire collection of gonococcal isolates, faropenem MIC testing using the CLSI defined supplement without L-cysteine resulted in a 4-fold lowering of the MIC_{50}, but only a 2-fold reduction of the MIC_{90} value (0.25 versus 0.5 µg/ml) compared to the commonly used method, i.e., GC agar based supplemented with 1.0% IsoVitaleX enrichment (Table 1).
- The rank order of antimicrobial potencies for the entire collection (265 isolates) was: ceftriaxone (MIC_{90}, 0.06 µg/ml) > faropenem (0.25 µg/ml) > azithromycin (0.5 µg/ml) > cefuroxime (1 µg/ml) > tetracycline (2 µg/ml) > penicillin = ciprofloxacin = levofloxacin (4 µg/ml; see Table 2). Using MIC_{90} results for comparison, faropenem was 4-fold more potent than cefuroxime (0.25 versus 1 µg/ml).
- Production of penicillinase (10.9% of collection) did not result in any adverse changes (Table 3) in the MIC results of the enzyme-stable ß-lactams tested.
- A majority of the gonococcal isolates (94.7%) had reduced susceptibility to penicillin (MIC, ≥ 0.12 µg/ml; see Table 3). Increasing resistance to penicillin was associated with elevated MIC_{50} and MIC_{90} values for faropenem and the four comparators analyzed. The rank order of the antimicrobials with the highest difference in the MIC_{90} values between the penicillin-susceptible and -resistant strains was: ciprofloxacin and cefuroxime (32-fold) > ceftriaxone and faropenem (16-fold) > tetracycline (8-fold; data not shown).
- More than one-third of the gonococcal isolates (39.6%) had reduced susceptibility to ciprofloxacin (Table 3), with 18.1% of strains tested having intermediate susceptibility (0.12-0.5 µg/ml) and 21.5% being resistant (≥ 1µg/ml). The activity of ß-lactams was not markedly affected by an increase in ciprofloxacin MIC values: for faropenem and cefuroxime there was only a 2-fold increase in the MIC_{90} value when comparing ciprofloxacin-susceptible and -resistant isolates.
- Analysis of 189 recent clinical isolates collected in 2004 from six distinct areas in the USA revealed that each faropenem MIC_{50} value (0.06 µg/ml) was identical to that obtained for the entire collection (265 strains; Table 3).
- There was a marked impact of the geographical site on the endemic antimicrobial susceptibility rates (Table 3). Penicillin susceptibility rates for the isolates from all six states were less than 20.0%, and ranged from 0.0% (New York) to only 16.7% (Oregon); and tetracycline susceptibility rates varied from 0.0% (New York) to 52.8% in Ohio (data not shown). Ciprofloxacin susceptibility rates varied markedly, ranging from 25.9% in Hawaii to greater than 90.0% in New York and Ohio (Table 3).
- The collection of 51 strains isolated from the Far East with some being characterized in Japan (31 strains with documented QRDR mutations) were analyzed separately (Table 4). Faropenem retained excellent activity with $MIC_{50/90}$ values of 0.12/0.25 µg/ml and with the highest MIC being only 0.25 µg/ml.

Table 1. Comparison of faropenem agar dilution MIC results obtained using the M7-A6 method and two growth supplements (with and without L-cysteine).

	MIC (µg/ml)		Cum. % inhibited at MIC (µg/ml)					
Supplement	50%	90%	≤ 0.03	0.06	0.12	0.25	0.5	1
A[a]	0.25	0.5	1.5	4.2	29.9	66.0	91.3	100.0
B[b]	0.06	0.25	26.0	58.1	88.3	99.6	100.0	-

a. A = supplement components of commercial IsoVitaleX containing high levels of L-cysteine. Cysteine can adversely effect the MIC results for carbapenems and clavulanate.
b. B = 1% defined supplement recommended by the CLSI agar dilution method M7-A6 and M100-S15.

Table 2. Faropenem activity compared to reference agents when tested against 265 isolates of *N. gonorrhoeae*

Antimicrobial agent	MIC (µg/ml) Range	50%	90%	% by category[a] Susceptible	Resistant
Azithromycin	0.03 - 2	0.25	0.5	-[b]	-
Ciprofloxacin	≤ 0.008 -> 4	0.008	4	60.4	21.5
Levofloxacin[c]	≤ 0.008 -> 4	0.016	4	61.9	21.1
Tetracycline	0.06 -> 4	0.5	2	20.4	24.2
Penicillin	0.016 -> 4	1	4	5.3	32.5
Ceftriaxone	≤ 0.008 - 0.12	≤ 0.008	0.06	100.0	-
Cefuroxime	≤ 0.008 - 2	0.25	1	95.8	0.0
Faropenem	≤ 0.008 - 0.5	0.06	0.25	-	-

a. Criteria for interpretation by the CLSI.
b. - = no published CLSI interpretive criteria.
c. Breakpoint criteria of ofloxacin were used corrected for the two-fold greater potency of levofloxacin (susceptible at ≤ 0.12 µg/ml; resistant at ≥ 1 µg/ml).

Table 3. Activity of faropenem tested against resistant subsets of *N. gonorrhoeae*

Resistance group (no. tested)	Faropenem MIC (µg/ml) Range	50%	90%	% ciprofloxacin susceptibility
Penicillinase				
Positive (PPNG; 29)	0.016 - 0.12	0.06	0.12	31.0
Negative (236)	≤ 0.008 - 0.5	0.06	0.25	64.0
Penicillin				
Susceptible (14)	≤ 0.008 - 0.016	0.016	0.016	85.7
Intermediate (165)	0.016 - 0.25	0.06	0.12	74.5
Resistant (86)	0.016 - 0.5	0.12	0.25	29.1
Ciprofloxacin				
Susceptible (160)	0.016 - 0.5	0.06	0.12	100.0
Intermediate (48)	0.016 - 0.25	0.12	0.25	0.0
Resistant (57)	0.03 - 0.25	0.12	0.25	0.0
States				
California (7)	0.016 - 0.25	0.06	-	71.4
Hawaii (27)	0.016 - 0.25	0.06	0.12	25.9
New York (25)	0.016 - 0.25	0.06	0.12	92.0
Ohio (36)	≤ 0.008 - 0.25	0.03	0.12	97.2
Oregon (18)	≤ 0.008 - 0.25	0.06	0.25	83.3
Washington (76)	≤ 0.008 - 0.25	0.06	0.12	65.8
All (189)	≤ 0.008 - 0.25	0.06	0.12	71.4
All strains (265)	≤ 0.008 - 0.5	0.06	0.25	60.4

Table 4. Activity of faropenem and selected comparison agents tested against a collection of *N. gonorrhoeae* isolates with documented fluoroquinolone resistances and mutations of QRDR (51 strains; from Asia-Pacific geographic region).

Antimicrobial agent	MIC (µg/ml) Range	50%	90%	% by category[a] Susceptible	Resistant
Faropenem	0.016 - 0.25	0.12	0.25	-[b]	-
Penicillin	0.03 -> 4	2	> 4	3.9	70.6
Ceftriaxone	≤ 0.008 - 0.12	0.03	0.12	100.0	-
Cefuroxime	0.06 - 2	0.5	2	86.3	0.0
Azithromycin	0.06 - 2	0.25	1	-	-
Tetracycline	0.5 - 4	1	2	0.0	35.3
Ciprofloxacin	0.12 -> 4	0.25	1	0.0	11.8
Levofloxacin[c]	0.12 - 4	0.25	1	5.9	11.8

a. Criteria for interpretation by the CLSI.
b. - = no published CLSI interpretive criteria.
c. Breakpoint criteria of ofloxacin were used corrected for the 2-fold greater potency of levofloxacin (susceptible at ≤ 0.12 µg/ml; resistant at ≥ 1 µg/ml).

CONCLUSIONS

- **Our results clearly confirmed that faropenem is affective *in vitro* against recent gonococcal isolates (MIC_{50}, 0.06 and MIC_{90}, 0.25 µg/ml). However, we observed that the activity of faropenem was adversely affected by L-cysteine hydrochloride in the medium supplement IsoVitaleX (4-fold increase in MIC_{50}).**
- **Despite the preselection bias towards antimicrobial-resistant gonococcal isolates (entire collection QRNG rate of 39.6%; high-level penicillin and tetracycline resistance rates of 32.5% and 24.2%, respectively), the potency of faropenem was competitive, and was positioned between ceftriaxone and cefuroxime.**
- **While the activity of faropenem and the other ß-lactams tested (ceftriaxone and cefuroxime) was not adversely affected by increasing ciprofloxacin MIC values, increasing penicillin MIC results were associated with an 8- to ≥ 32-fold increase in the MIC_{90} value of faropenem and some comparators.**
- **In the analysis of recent clinical gonococcal isolates from six states in the USA (Table 3), the reduced susceptibility rates to fluoroquinolones, tetracycline and penicillin were very high (≥ 28.0%). Despite these high rates of fluoroquinolone resistance, ceftriaxone, cefuroxime and faropenem remained active against these isolates (ceftriaxone 100.0% susceptible, faropenem MIC_{90}, ≤ 0.25 µg/ml).**
- **Our results indicate that faropenem has excellent activity against this collection of gonococcal isolates analyzed, regardless of the resistance mechanism or phenotype. The potency of faropenem was comparable to preferred therapeutic ß-lactams and based on its microbiologic performance characteristics, faropenem could join ceftriaxone and some orally administered cephalosporins as possible alternatives.**

ACKNOWLEDGMENTS

The co-authors express their gratitude to the following contributors to this investigation: L. Deshpande, D.J. Biedenbach, M.G. Stilwell and K.L. Meyer. We would also like to thank Joseph DiPersio (Summa Health Systems), Dwight Hardy (University of Rochester Medical Center), Judy Fusco (TPMG Regional Laboratory-Berkeley) and William L.H. Whittington (University of Washington) for their assistance in the collection of recent clinical isolates. This study was sponsored by an educational/research grant by Replidyne, Inc.