



Neue Beta-Lactame und Beta-Lactamase-Inhibitoren

Neue Beta-Lactame

Code	Name	Gruppe	Wirksam gegen	Abstract
BAL30072	--	Monobactam	Gram(-) Non-Fermenter	F1-1164 ; F1-1165 ; F1-1166 ; F1-1167
BAL30376	--	Monobactam	<i>Ps. aeruginosa</i>	F1-1166
CS-023	Tomopenem	Carbapenem	<i>Ps. aeruginosa</i>	A-026 ; A-027
CXA-101 (FR264205)		Cephalosporin	<i>Ps. aeruginosa</i>	F1-354 ; F1-355 ; F1-356 ; F1-357 ; F1-358
FOV	Cefovecin	Cephalosporin	Gram (+/-) bei Kleintieren	D-2228
ME-1036	--	Carbapenem	Gram(+/-) Bakterien	F1-343
SMP-601	PZ-601	Carbapenem	Anaerobier; Gram(+/-) Bakterien	C1-099 ; C1-102 ; C1-195 ; C2-3930 ; F1-359 ; F1-360 ; F1-361
--	Ceftarolin	Cephalosporin	Gram(+) Bakterien	A-979 ; A-991 ; B-068 ; B-1003 ; C1-160 ; C1-161 ; C1-162 ; C1-163 ; C1-182 ; C1-183 ; C1-185 ; C2-255 ; C1-3719 ; C2-1974 ; D-2232 ; D-2249
--	Faropenem	Carbapenem	Gram(+/-) Bakterien	L-662a
--	Sulopenem	Carbapenem	Gram(+/-) Bakterien	A-054 ; F1-344 ; F1-345 ; F1-346 ; F1-347 ; F1-348 ; F1-349 ; F1-350 ; F1-351 ; F1-352 ; F1-353
PF-03709270	Sulopenem-Prodrug	Carbapenem	Gram(+/-) Bakterien	F1-353

Neue Beta-Lactamaseinhibitoren

Code	Name	Gruppe	Wirksam gegen	Abstract
BLI-489	--	Carbapenemase-inhibitor	Klasse A, C, D beta-Lactamasen	F1-1168 ; F1-1169
ME1071	--	Metallo-beta-Lactamaseinhibitor	MBL-Bildner	F1-1170 ; F1-1171
NXL104	--	Non-beta-Lactam	<i>Klebsiella spp.</i> <i>Enterobacter spp.</i>	A-023 ; D-291b ; F1-1172

A-023

Importance of NXL104 Pharmacokinetics (PK) in the Pharmacodynamics (PD) of Ceftazidime+NXL104 Combinations in an In Vitro Hollow Fiber Infection Model

M. BORGONOV, H. MERDJAN, A. GIRARD, P. LEVASSEUR, M. QUERNIN, J. LOWTHER, C. MIOSSEC, D. SHLAES; Novexel S.A., Romainville, France.

Background: NXL104 is a novel β -lactamase inhibitor that restores ceftazidime (CAZ) activity against CAZ-resistant strains. CAZ pharmacodynamics is Time>MIC dependent, but little is known about β -lactam+ β -lactamase inhibitor PK/PD relationship. The aim of the study was to determine the importance of NXL104 PK in the PD of CAZ+NXL104 combinations.

Methods: An *in vitro* hollow fiber infection model was used to expose exponentially growing cultures of four *Enterobacteriaceae* strains to two different dosing regimens of the combination: 1) CAZ + NXL104 continuous infusion 2) CAZ + NXL104 human-like profile (mimicking a bi-exponential profile following a single 30 min intravenous administration in humans) CAZ was set constant at 16 mg/L during the entire test period. NXL104 was given so as to have the same total exposure in the two regimens, but with different concentration-time curves. Samples were taken at different time points for determination of viable bacterial count and CAZ and NXL104 concentrations.

Results: The combination CAZ + NXL104 was rapidly cidal against *E. cloacae* 293HT96 (AmpC), *K. pneumoniae* (K.p.) Tunisie C4 (CTX-M-15), *K.p.* 181 and *K.p.* 236 (SHV-5, TEM-10), reducing the bacterial count by 3 log₁₀ within 4h. Growth of the four strains was fully suppressed throughout the test period following the continuous infusion regimen (1), while antibacterial effect of the combination was lost when the concentration of NXL104 fell below a critical level, in these experimental conditions <0.5 μ g/ml, as seen after exposure to the human-like profile (2).

Conclusions: These experiments have demonstrated qualitatively that one of the most important factors affecting the PD of the CAZ+NXL104 combination in this experimental setting is the maintenance of a critical concentration of inhibitor necessary to sufficiently suppress β -lactamase activity.

A-026

In Vivo Efficacy of Tomopenem (formerly CS-023) Human-Simulated Exposure Against *Pseudomonas aeruginosa* and MRSA

K. TATEDA ¹, K. SUGIHARA^{2,1}, N. YAMAMURA ², T. KOGA ², C. SUGIHARA ², K. YAMAGUCHI ¹;
¹Toho Univ. Sch. of Med., Tokyo, Japan, ²Daiichi Sankyo Co., Ltd., Tokyo, Japan.

Background: Tomopenem is a novel carbapenem with broad-spectrum activity against diverse hospital pathogens, including *Pseudomonas aeruginosa* and MRSA, and a longer half-life. In this study, we evaluated the *in vivo* efficacy of tomopenem human-simulated exposure against *P. aeruginosa* compared with meropenem (MEPM) and MRSA.

Methods: The *in vivo* efficacy was assessed in a neutropenic murine thigh infection model against 9 clinical isolates of *P. aeruginosa* (MICs, 4-32 µg/mL) and 9 clinical isolates of MRSA (MICs, 4-16 µg/mL) for tomopenem, and 9 clinical isolates of *P. aeruginosa* (MICs, 2-16 µg/mL) for MEPM. Human-simulated dosing regimens in neutropenic mice were designed to approximate the free time above MIC (*fT*>MIC) observed with tomopenem at 750 mg TID and 1500 mg TID and MEPM at 1000 mg TID given as 0.5 h infusion in humans, respectively.

Results: In this model, tomopenem 750 mg TID showed bactericidal or bacteriostatic effects for 5 strains of *P. aeruginosa* with MICs ≤8 except for 1 of 3 with MICs = 8, and all 6 strains of MRSA with MICs ≤8. Tomopenem 1500 mg TID showed bactericidal effects for all strains of *P. aeruginosa* and MRSA except for 2 strains with MICs = 16 and 32 of *P. aeruginosa*. In the case of MEPM 1000 mg TID, it showed bactericidal or bacteriostatic effects against 4 strains of *P. aeruginosa* with MICs ≤4 except for 1 of 3 with MICs = 4, while regrowth was observed against 5 strains with MICs ≥8, except for 1 of 2 with MICs = 16. This seemed to almost correlate with the CLSI-defined breakpoint. As is generally the case for carbapenems, the pharmacodynamic target of tomopenem against *P. aeruginosa* was considered to be *fT*>MIC of 30-40% by a sigmoid dose-effect model.

Conclusions: Tomopenem demonstrated good *in vivo* efficacy in human-simulated exposure against *P. aeruginosa* and MRSA and is expected to be effective at over 40% *fT*>MIC which is obtained at MIC ≤8 µg/mL for 750 mg TID and MIC ≤16 µg/mL for 1500 mg TID.

A-027

In Vivo Pharmacodynamic Activity of Tomopenem (formerly CS-023) Against *Pseudomonas aeruginosa* and MRSA in Murine Thigh Infection Model

K. SUGIHARA, Y. MATSUSHITA, N. YAMAMURA, M. UEMORI, C. SUGIHARA, A. TOKUMITSU, H. INOUE, M. KAKUTA, E. NAMBA, H. NASU, T. KOGA;
Daiichi Sankyo Co., Ltd., Tokyo, Japan.

Background: Tomopenem is a novel carbapenem with broad-spectrum activity against diverse hospital pathogens, including *Pseudomonas aeruginosa* and MRSA, and a longer half-life. In this study, we evaluated the *in vivo* pharmacodynamic activity of tomopenem against *P. aeruginosa* and MRSA.

Methods: The *in vivo* efficacy was assessed in a neutropenic murine thigh infection model with *P. aeruginosa* 12467 (MIC, 1 µg/mL) and MRSA 12372 (MIC, 2 µg/mL). Neutropenia was induced by 150 and 100 mg/kg cyclophosphamide at 4 days and 1 day before inoculation, respectively. The mice had 10^{6-7} CFU/thigh of the 2 strains 2 hr after inoculation, and were treated for 24 hr with fractionated administration of tomopenem given at 3, 6, 12, and 24 hr intervals. Single-dose serum concentrations were determined in the same model with *P. aeruginosa*. A sigmoid dose-effect model was used to estimate the magnitude required for a static effect, 1-log kill, and 2-log kill in CFU/thigh.

Results: The efficacy of tomopenem was enhanced by frequent dosing, which indicates that the efficacy is driven by the time above MIC (T>MIC). The strongest relationships were observed when the results were correlated with the free T>MIC ($fT>MIC$) with an $R^2=0.79$ (AUC/MIC R^2 , 0.42; C_{max}/MIC R^2 , not calculable) in the *P. aeruginosa* infection model and with an $R^2=0.86$ (AUC/MIC R^2 , 0.33; C_{max}/MIC R^2 , not calculable) in the MRSA infection model. The values of % $fT>MIC$ required for a static effect, 1-log kill, and 2-log kill were 29, 39, and 51, against *P. aeruginosa*, respectively, and they were similar against MRSA with values of 26, 34, and 46.

Conclusions: The magnitude of $fT>MIC$ of tomopenem required for efficacy is similar to other carbapenems and there is no difference between *P. aeruginosa* and MRSA. Considering the almost two times longer elimination half-life of tomopenem, it is expected to be more effective than other carbapenems against diverse hospital pathogens.

A-054

Pharmacokinetics and Pharmacodynamics of Sulopenem in Preclinical Species

D. GIRARD, S. M. FINEGAN, J. P. O'DONNELL;
PGRD Groton/New London Labs, Groton, CT.

Background: The pharmacokinetics (PK) and pharmacodynamics (PD) of Sulopenem, a thiopenem with potent activity against gram-positive and gram-negative bacteria, were characterized in neutropenic mouse thigh infections against clinical strains of *S. pneumoniae* and *K. pneumoniae* and fitted to an Emax model. Non-linear regression analysis was used to determine the correlation between PK/PD parameters and reduction in bacterial CFU/thigh at 24 hrs.

Methods: Murine thigh infections were established in neutropenic CF-1 mice, subcutaneous therapy was initiated 1 h following challenge covering a 64-fold dose range administered every q3h, q6h, q12h or q24h. The bacterial burden in tissues was determined after 24 h of therapy using standard techniques. Single dose PK studies were conducted and plasma levels determined using an LC/MS/MS assay with PK parameters estimated using non-compartmental methods. The 24 h PD parameters were estimated from PK parameters corrected for free fraction and static doses and ED₉₀ values estimated after fitting the data to an E_{max} model. The correlation between efficacy and PK/PD parameters was determined by nonlinear least-squares multivariate regression.

Results: The pharmacokinetic parameters for Sulopenem over the dose range of 6.3-200 mg/kg were: C_{max} values ranged from 1.9-51 µg/ml, and AUC₀₋₂₄ ranged from 1.1-28 µg-h/ml. Static doses ranged from 4-54 and 5-56 mg/kg/day for *S. pneumoniae* and *K. pneumoniae*, respectively. The goodness of fit for the 24 h PD parameters were an R² of 0.84 for time above MIC, 0.45 for AUC/MIC and 0.45 for Cmax/MIC. The time above MIC (fu) for the ED₉₀ was 18% and 39% of the dosing interval vs. *S. pneumoniae* and *K. pneumoniae*, respectively.

Conclusion: 1) Dose-proportional pharmacokinetics was demonstrated with Sulopenem. 2) Sulopenem was active against penicillin-resistant *S. pneumoniae* and ESBL *K. pneumoniae* in these models. 3) Time above MIC was the PD parameter most closely linked with outcome for Sulopenem and is consistent with what is reported for carbapenems and penems.

A-979

In Vitro Activity of Ceftaroline (CPT) vs. Vancomycin (VM) Against MRSA and *h*VISA Strains in a Pharmacokinetic/pharmacodynamic (PK/PD) Model

C. VIDAILLAC, S. N. LEONARD, M. J. RYBAK;
Wayne State Univ., Detroit, MI.

Background: With the increased use of VM and the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to glycopeptides, the discovery of new anti-MRSA agents is a current worldwide concern. CPT is a novel broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including MRSA, *h*VISA and multidrug-resistant *Streptococcus pneumoniae*, as well as common gram-negative pathogens. Using an *in-vitro* PK/PD model, we evaluated CPT activity vs. VM against 3 clinical MRSA: 494 and 3804 (MRSA); 1629 (*h*VISA).

Methods: MICs and MBCs were determined per CLSI guidelines. CPT and VM activity were evaluated for 72 h using a 2-compartment Hollow Fiber model to simulate free drug concentrations. CPT (600-1200 mg q6-12h) and VM (1000 mg q12h) were studied with a starting inoculum from $\sim 10^6$ to $\sim 10^8$ CFU/mL. We present results of the CPT regimen at 600 mg every 8h with a starting inoculum of $\sim 10^7$ CFU/mL. Changes in CFU/mL at 72 h were evaluated by t-test.

Results: CPT exhibited an MIC range of 0.125-0.5 mg/L, whereas the VM MIC range was 0.5-2 mg/L. Achieved PK parameters were $T_{1/2}$ of 8 and 2h, and free peaks of 18 mg/L and 15 or 30 mg/L for VM and CPT, respectively. In the PK/PD model, CPT (600 mg) and VM activity were similar against 494 ($MIC_{CPT}=MIC_{VM}=0.5$ mg/L). Bacterial regrowth, uncorrelated to emergence of resistance, instability of the drug or tolerance, was observed after 32h and slightly reduced with high dose. In contrast, CPT showed significantly greater activity compared to VM against 1629 and 3804 ($MIC_{CPT}=0.125$ and 0.25 mg/L, $MIC_{VM}=2$ and 0.5 mg/L, respectively) ($P<0.01$). Finally, emergence of resistance was observed with VM at 72 h for the *h*VISA strain.

Conclusions: CPT *in-vitro* activity was similar or superior to VM against MRSA and superior to VM against the *h*VISA isolate. In addition to its low potential to select resistant mutants, CPT may be a promising alternative for the treatment of MRSA infection.

A-991

Comparative Pharmacokinetics (PK) of Ceftaroline (CPT) in Rats, Rabbits, and Monkeys Following a Single Intramuscular (IM) or Intravenous (IV) Injection

Y. GE¹, D. MAYNARD², D. E. RICKERT³;

¹Cerexa, Inc., Alameda, CA, ²Lab Rsearch, Inc., Laval, Canada, ³Douglas E. Rickert, LLC, Raleigh, NC.

Background: Ceftaroline (CPT) is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin -resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common gram-negative pathogens. CPT is currently in phase 3 development. The prodrug, CPT fosamil, is rapidly dephosphorylated into bioactive CPT. To explore the utility of CPT for IM administration, the PK profiles of CPT were evaluated in rats, rabbits, and monkeys following a single IM or IV injection.

Methods: Animals received CPT 20 mg/kg as either a 1-minute IV infusion or a single IM injection. In rabbits and monkeys, the crossover study design included a washout period between IM and IV dosing. Samples were analyzed by a validated bioanalytical method for CPT. The area under the plasma concentration-time curve ($AUC_{0-\infty}$), terminal half-life ($t_{1/2}$), highest plasma concentration observed (C_{max}), and time to C_{max} (T_{max}) of CPT were determined.

Results: $AUC_{0-\infty}$ was higher after IM dosing than after IV dosing in all 3 animal species, indicating excellent IM bioavailability. The $t_{1/2}$ was longer in rats and rabbits and similar in monkeys (IM vs IV). C_{max} values were lower for IM dosing (vs IV).

Conclusions: CPT demonstrated excellent bioavailability and favorable PK profiles after IM dosing in all 3 animal species, which supports the clinical development of CPT for IM administration for treating bacterial infections in the hospital setting as well as outside of the hospital.

A-991 (Forts.)

PK parameters for CPT after IM or IV dosing

Parameter	Rat (N=18) ^a	Rabbit (N=4)	Monkey (N=4)
IM			
AUC _{0-∞} (h·ng/mL)	37,400	39,700	72,100
C _{max} (ng/mL)	25,500	25,800	25,600
t _{1/2} (h)	0.621	0.833	1.17
T _{max} (h)	0.500	0.438	0.500
IV			
AUC _{0-∞} (h·ng/mL)	16,300	37,000	64,000
C _{max} (ng/mL)	NR	67,800	69,100
t _{1/2} (h)	0.426	0.410	1.16
T _{max} (h)	0.083	0.083	0.083

NR = not reported.

^aN=9 for IM dose; N=9 for IV dose.

B-068

Assessment of the In Vivo Activity of Ceftaroline (CPT) Against Vancomycin-Susceptible and -Resistant *Enterococcus faecalis* (EF) Strains in a Rabbit Endocarditis Model (REM): Comparison with Linezolid (LZO) and Vancomycin (VAN)

C. JACQUELINE¹, G. AMADOR¹, E. BATARD¹, V. LE MABECQUE¹, D. BIEK², J. Y. GE², G. POTEL¹, J. CAILLON¹;
¹UPRES EA 3826, Nantes, France, ²Cerexa, Inc., Alameda, CA.

Background: Ceftaroline, the bioactive metabolite of ceftaroline fosamil, is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Streptococcus pneumoniae* (MDRSP), and VAN-resistant EF, as well as common gram-negative pathogens. CPT is currently in phase 3 development. The objective of this study was to evaluate and compare the in vivo activity of CPT with LZO and VAN against EF strains using simulated human dosing in a REM.

Methods: MICs for EF 12704 and EF NJ1 were 2 and 1 mg/L for CPT, 2 and >256 mg/L for VAN, and 2 and 1 mg/L for linezolid, respectively. The pharmacokinetics of CPT and comparators were determined to facilitate human dose simulation. Animals infected with one of the two EF strains were randomly assigned to no treatment (controls), CPT (computer-controlled infusion syringe pump simulating a human-equivalent (HE) dosage of 10mg/kg/12h), LZO (simulating an HE dosage of 10mg/kg/12h), and VAN (administered by a constant IV infusion to reach 20xMIC steady-state concentration). Surviving bacteria were counted in vegetations (Veg.) at the end of 4 days of treatment.

Results: (see table)

Conclusions: CPT exhibited significant in vivo activity against both EF strains. This novel cephalosporin was significantly more active than LZO and VAN against VAN-S and VAN-R EF. The in vivo activity of CPT against EF appears to be related to the MIC of the strain.

Treatment	Mean ± SD log ₁₀ CFU/g of Veg.	
	EF 12704 Van-S (n)	EF NJ1 Van-R (n)
Controls	8.56 ± 0.74 (8)	8.60 ± 0.54 (9)
CPT (HE 10mg/kg/12h)	5.68 ± 0.49 (7) ^{a, d}	3.98 ± 0.85 (9) ^{a, b}
LZO (HE 10mg/kg/12h)	6.88 ± 0.70 (7) ^a	6.88 ± 0.77 (9) ^{a, c}
VAN	6.70 ± 0.25 (8) ^a	8.01 ± 0.76 (8)

(n): (number of animals). a: $P < 0.001$ vs controls; b: $P < 0.001$ vs LZO and VAN; c: $P < 0.05$ vs VAN.

B-1003

Evaluation of the Efficacy of Intramuscular (IM) Administration of Ceftaroline (CPT) Against a Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strain in a Rabbit Endocarditis Model (REM)

C. JACQUELINE¹, J. CAILLON¹, V. LE MABECQUE¹, E. BATARD¹, A. F. MIEGEVILLE¹, D. BIEK², J. Y. GE², G. POTEL¹; UPRES EA 3826, Nantes, France, ²Cerexa, Inc., Alameda, CA.

Background: Ceftaroline is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including MRSA and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common gram-negative pathogens. CPT is currently in phase 3 development. We previously reported the highly bactericidal activity of IV CPT against MRSA in rabbit. The objective of this study was to compare the activity of 3 different doses of CPT with teicoplanin (TEC) after IM administration against a MRSA strain using a REM.

Methods: MICs were 1 and 0.5 mg/L for CPT and teicoplanin (TEC), respectively. Preliminary PK studies were performed to determine the PK parameters of IM administration and facilitate dose selection. Animals were randomized to: no treatment (controls), CPT 5 mg/kg bid, CPT 20 mg/kg bid, CPT 40 mg/kg bid, or TEC 20 mg/kg bid.

Results: The absolute bioavailability of IM CPT exceeded 90% of IV CPT (as measured by AUC). The C_{max} of CPT at 5, 20, and 40 mg/kg increased approximately in proportion to dose (5.18, 15.75, and 37.85 mg/L, respectively). The plasma elimination half-life was 0.74-1.14 hours over the range tested. Bacterial titers in vegetations (Veg.) after 4 days of treatment were.

Conclusions: IM CPT demonstrated excellent bactericidal activity against the MRSA strain at 20 and 40 mg/kg, resulting in sterilization rates in vegetations of 80% and 100%, respectively. The results strongly support IM CPT as a promising and effective therapeutic option for the treatment of severe MRSA infections.

Regimen	Mean ± SD log ₁₀ CFU/g of Veg. (n)
Controls	8.99 ± 0.47 (0/10)
IM CPT (40 mg/kg bid)	2.45 ± 0.14 (10/10) ^{a,c}
IM CPT (20 mg/kg bid)	3.14 ± 1.38 (8/10) ^{a,b}
IM CPT (5 mg/kg bid)	5.26 ± 2.73 (3/9) ^a
IM TEC (20 mg/kg bid)	3.07 ± 0.66 (6/10) ^{a,b}

(n): no. of sterile veg./total no. of veg.; a: $P < 0.001$ vs controls; b: $P < 0.05$ vs IM CPT (5 mg/kg bid); c: $P < 0.001$ vs IM CPT (5 mg/kg bid); Bonferroni's test after analysis of variance.

C1-099

In Vitro Activity of PZ-601 (SMP-601) and Comparators Against 203 isolates of *Bacteroides fragilis* group and 71 Toxigenic *Clostridium difficile* Clinical Isolates

D. W. HECHT^{1,2}, D. N. GERDING², J. R. OSMOLSKI¹; ¹Loyola Univ. Med. Ctr., Maywood, IL, ²Hines VA Hosp., Hines, IL.

Background: PZ-601 is a new carbapenem active against multi drug resistant gram-positive and gram-negative pathogens. We have evaluated the activity of PZ-601 against anaerobic bacteria, including isolates of *C. difficile*.

Methods: 203 recent clinical isolates of the *B. fragilis* group were tested against PZ-601, piperacillin/tazobactam (PTZ), clindamycin (CLN), metronidazole (MET), and imipenem (IMI) and 71 unique toxigenic *C. difficile* isolates of differing REA types known to have caused infection were tested against PZ-601, PTZ, MET, IMI, and vancomycin (VAN). Inoculum preparation and agar dilution were performed according to the CLSI method for anaerobes (M11-A7).

Results: PZ-601 activity against members of the *B. fragilis* group was most similar to that of IMI with identical MIC₅₀ and MIC₉₀ values of 0.25 µg/mL and 1 µg/mL, respectively, although three isolates of *B. fragilis* showed MICs > 8 µg/mL compared with none for IMI (Table). PZ-601 was the most active of all five agents against the *C. difficile* isolates with MIC₅₀ and MIC₉₀ values of 1 µg/mL and 2 µg/mL, respectively.

Conclusions: The *in vitro* activity of PZ-601

is comparable to or more active than antibiotics used to treat anaerobic infections that involve members of the *B. fragilis* group, and warrants clinical trials to establish efficacy. The excellent activity of PZ-601 against isolates of *C. difficile* may be important in patients treated with this antibiotic and otherwise at risk for *C. difficile* colitis providing a potential protective benefit while being given. Clinical trials of PZ-601 and comparators should include incidence of *C. difficile* as a secondary endpoint.

Drug	<i>Bacteroides fragilis</i> group			<i>C. difficile</i>		
	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
PZ-601	≤0.015-32	0.25	1	0.5-4	1	2
IMI	≤0.015-4	0.25	1	2-8	4	8
PTZ	≤0.06->64	4	8	2-16	8	8
MET	0.25-2	1	1	0.125-2	0.25	1
CLN	≤0.03->64	4	>64	----	----	----
VAN	----	----	----	0.5-4	1	2

C1-102

Effect of PZ-601 (smp-601) on Genotypically Characterized *Staphylococcus aureus* (MRSA) Representing the Major Epidemic Clones

S. BORBONE ¹, D. BONGIORNO ¹, J. SAFAA ², G. MONGELLI ¹, C. SCUDERI ¹, F. CAMPANILE ¹, **S. STEFANI**¹;

¹Univ. of Catania, Catania, Italy, ²Univ. of Catania - Scuola Superiore, Catania, Italy.

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is still one of the most feared hospital and, recently, community-acquired pathogens. Continuously challenged by therapies with diverse classes of antibiotics, this species has become less susceptible by elaborating different strategies. PZ-601 is a new broad spectrum parenteral carbapenem and previously published results show promising bactericidal activity against contemporary MDR strains of MRSA.

Methods: The resistance mutation rate to PZ-601 was evaluated in the Archaic, Iberian, Italian, Brazilian, Rome heteroGISA, C-MRSA epidemic clones, by using fluctuation analysis at 4x, 8x and 16x MIC concentrations of antibiotic, and a multi-step generation of mutants after serial passages in the presence of sub-inhibitory concentrations of the drug. The identity of the clones was confirmed by PFGE

Results: No change in susceptibility to PZ-601 was observed with all 10 different MRSA clones following 15 passages at sub-inhibitory concentrations.

Fluctuation analysis with the Archaic, Iberian, Brazilian and Roman clones did not detect any mutant, out of 10^{10} cells, resistant to 4x MIC of PZ-601. The hGISA and the Italian clones demonstrated approximately 1×10^{-7} - 10^{-8} mutation rate at 4x MIC of PZ-601. Only the hGISA mutants reverted to sensitivity after passaging in antibiotic-free medium.

Conclusion: Development of resistance to PZ-601 was extremely low with any of the MRSA clones used in this study under the pressure of continuous passages at sub-inhibitory drug concentrations. Applying the fluctuation method, 4x MIC was sufficient to prevent occurrence of any mutation in the Archaic, Iberian, Brazilian and Roman clones and 8x MIC was needed with the h-GISA and the Italian clones.

C1-160

Ceftaroline Activity Tested Against Organisms Causing Skin and Skin Structure Infections (SSSI) Isolated in USA and European Medical Centers in 2008

R. N. JONES, T. R. FRITSCHKE, H. S. SADLER; JMI Lab., North Liberty, IA.

Background: Ceftaroline (CPT) is the bioactive metabolite of ceftaroline fosamil, a N-phosphonoamino water-soluble cephalosporin prodrug. CPT is active against oxacillin-resistant *S. aureus* (MRSA) and is under evaluation for treatment of SSSI in clinical trials. We assessed the activity of CPT and comparator agents tested against SSSI pathogens.

Methods: Non-duplicate clinically-significant strains of *S. aureus* (SA; 1517) and β -haemolytic streptococci (β HS; 157) were consecutively collected from more than 50 medical centers in the USA and Europe (EU) and susceptibility (S) tested by CLSI broth microdilution methods (M7-A7; M100-S18) against CPT and >20 antimicrobials currently available for SSSI treatment.

Results: 49.9% of SA was MRSA. Over 60% of SA strains were from community origin and showed MRSA rate similar to health-care associated SA. CPT was very active against oxacillin-S SA (MSSA; MIC₉₀, 0.25 μ g/ml) and MRSA (MIC₉₀, 1 μ g/ml). Against MSSA, CPT was 16- and four-fold more potent than ceftriaxone and vancomycin, respectively. 95.7 and 99.9% of MSSA were inhibited at £0.25 and £0.5 μ g/ml, respectively (highest CPT MIC was 1 μ g/ml). The % of MRSA inhibited at 1 and 2 μ g/ml of CPT were 96.7 and 100.0% respectively. β HS was very S to CPT and all strains were inhibited at £0.03 μ g/ml.

Conclusions: CPT showed high potency (MIC₉₀ range, 0.015-1 μ g/ml) and broad spectrum against contemporary (2008) SSSI Gram-positive pathogens, including MRSA and fluoroquinolone resistant β HS, isolated in USA and EU medical centers. Based on these results, CPT appears to be a promising agent for the treatment of organisms causing SSSI.

Organism (no.)	MIC ₉₀ (mg/ml)/ % S					
	ceftaroline	ceftriaxone	clindamycin	levofloxacin	co-trimoxazole	vancomycin
MSSA (761)	0.25/NA ^a	4/99.3	≤ 0.25/94.0	2/89.5	≤0.5/98.8	1/100.0
MRSA (756)	1/NA ^a	>32/0.0	>2/70.4	>4/29.4	≤ 0.5/98.9	1/100.0
β HS (157)	0.015/NA ^a	≤ 0.25/100.0	≤ 0.25/91.7	1/99.4	≤ 0.5/-	0.5/100.0

a. NA = not assigned.

C1-161

***In Vitro* Activity of Ceftaroline (CPT) in Combination Against Extended-Spectrum β -lactamase (ESBL) Producing Gram-Negative (GN) Bacteria**

C. VIDAILLAC¹, S. N. LEONARD¹, H. S. SADER², R. N. JONES², M. J. RYBAK¹;

¹Wayne State Univ., Detroit, MI, ²JMI Lab., Inc., North Liberty, IA.

Background: CPT is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Streptococcus pneumoniae*, as well as common GN pathogens. The activity of CPT cannot be solely relied upon for effective eradication of ESBL producing Enterobacteriaceae. Drug combinations might be beneficial by increasing the susceptibility of these pathogens. We evaluated the activity of CPT combined with meropenem (MEM), piperacillin-tazobactam (P/T), cefepime (CPM), amikacin (AMK), levofloxacin (LEV), aztreonam (AZ) and tigecycline (TGC).

Methods: MICs and MBCs were determined per CLSI guidelines against 20 PSA, 10 ESBL producing *Escherichia coli* (*Ec*), 10 ESBL producing *Klebsiella pneumoniae* (*Kp*), and 10 AmpC-derepressed *Enterobacter cloacae* (*Ecl*). Eight isolates were randomly selected for time kill experiments (TK) with drug combinations at ¼ MIC. Synergy (>2 log₁₀ kill), additive effect (<2 but >1 log₁₀ kill), antagonism (>1 log₁₀ growth) and indifference (\pm 1 log₁₀ kill) were assessed.

Results: CPT exhibited an MIC range of 2-1024 μ g/ml, except for 4 *Ecl* (MIC \leq 1 μ g/ml). MBCs were similar to 2-fold higher than MICs. In TK, no antimicrobial alone was cidal at ¼ MIC. Whereas CPT plus TGC, LEV or CPM were mainly indifferent, CPT plus AMK was synergistic against all isolates. CPT plus P/T appeared synergistic against *Ec* and *Kp*, but indifferent for *Ecl* and indifferent/additive for PSA. CPT plus MEM or AZ was synergistic against *Ec* or *Ecl*, respectively, but indifferent against all other isolates except for 1 PSA (additive). No CPT antagonism was observed.

Conclusions: CPT plus AMK was active and appeared to be synergistic against all isolates studied. Other combinations yielded mixed results. Further study of CPT in combination is warranted to elucidate its clinical value.

C1-162

***In Vitro* Activity of Ceftaroline Against CA-MRSA, VISA, VRSA and Daptomycin-Non-Susceptible *Staphylococcus aureus* (DNSSA)**

L. D. SARAVOLATZ, J. PAWLAK, L. JOHNSON; St John Hosp. and Med. Ctr., Grosse Pointe Woods, MI.

Background: Ceftaroline (CPT) is novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin-resistant-*Staphylococcus aureus* (MRSA), multidrug-resistant *Streptococcus pneumoniae* and common gram-negative pathogens. CPT is currently in phase 3 development. This study evaluated the *in vitro* activity of CPT against isolates of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *Staphylococcus aureus* (VRSA) and DNSSA.

Methods: MIC values were determined by microdilution assay according to CLSI guidelines for CPT and 6 comparators for 92 CA-MRSA, 23 VISA, 8 VRSA, and 6 DNSSA isolates. Minimal bactericidal concentrations (MBC) were determined using CLSI methods. Pulse-field gel electrophoresis of CA-MRSA isolates was performed using SmaI restriction endonuclease digestion. Staphylococcal cassette chromosome (SCC) *mec* typing was determined by multiplex-polymerase chain reaction (PCR). Panton-Valentine leukocidin (*pvl*) genes were identified by PCR.

Results: see table. CAMRSA: 43% *pvl*(+), 57% SCCType IV, 41% SCCType II, VISA: 100% *pvl*(-), 91% SCCType II.

Conclusions: Ceftaroline demonstrated the greatest overall *in vitro* bactericidal activity against VISA, VRSA and DNSSA isolates and may represent a promising treatment for infections involving resistant *S. aureus* when compared to vancomycin, daptomycin, clindamycin, linezolid, TMP/SMX and ceftriaxone. Against CA-MRSA ceftaroline was equal in activity to vancomycin and daptomycin and superior to clindamycin, linezolid, TMP/SMX and ceftriaxone.

	CAMRSA (n=92)	VISA (n=23)	DNSSA (n=6)	VRSA (n=8)
	MIC 90 (MBC 90/MIC90)	MIC 90 (MBC 90/MIC90)	MIC Range (MBC Range)	MIC Range (MBC Range)
Ceftaroline	1 (1)	1 (1)	0.5-1 (0.5-1)	0.12-1 (0.12-1)
Vancomycin	1 (1)	8 (1)	2 (2)	32->64 (64->64)
Daptomycin	1 (1)	4 (1)	4 (4-8)	0.5-1 (0.5-1)
Clindamycin	>64 (1)	>64 (1)	<0.03->64 (2->64)	>64 (>64)
Linezolid	2 (>4)	2 (>4)	1-2 (2->8)	1-4 (8->8)
TMP/SMX	0.5/9.5 (2)	>4/76 (1)	0.12/2.4->4/76 (0.12/2.4->4/76)	0.06/1.2-2/38 (0.12/2.4->4/76)
Ceftriaxone	>64 (1)	>64 (1)	32->64 (64->64)	64->64 (>64)

C1-163

***In Vitro* activity of Ceftaroline (CPT) Against Recent US Isolates of *Neisseria gonorrhoeae* (NG)**

P. M. BARRY¹, C. J. LENDERMAN ², J. H. MELENDEZ ³, W. H. WHITTINGTON ⁴, E. J. HOOK ², J. M. ZENILMAN ³, J. D. KLAUSNER ¹; ¹UCSF, San Francisco, CA, ²UAB, Birmingham, AL, ³Johns Hopkins, Baltimore, MD, ⁴U Washington, Seattle, WA.

Background: Antibiotic resistance of NG is an emerging public health problem. Resistance to multiple antibiotic classes has been documented, including to quinolones and cephalosporins such as cefixime and ceftriaxone. CPT is a novel parenteral cephalosporin in phase 3 development with bactericidal activity against gram-positive and gram-negative organisms including methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Streptococcus pneumoniae*. This study assessed CPT's activity against recent NG isolates from the United States.

Methods: NG isolates collected during 2005-2007 and selected to demonstrate a spectrum of clinically relevant resistance patterns were tested with CPT and 7 comparators using CLSI agar-dilution methods.

Results: Of 404 NG isolates, 87% were from males; 71% urethral, 10% vaginal/cervical, 9% pharyngeal and 9% rectal. Isolates were from Baltimore (31%), U. Washington laboratory (26%), Philadelphia (19%), Oklahoma City (10%), Birmingham (8%), and Cincinnati (6%). Isolate collections included organisms with plasmid, chromosomal and combined resistance to β -lactams. All isolates were susceptible to spectinomycin, 11 isolates (3%) produced β -lactamase, and 128 (32%) had reduced susceptibility to ciprofloxacin. MIC50, MIC90 and ranges are shown (Table).

Conclusions: As measured by MIC90, CPT had greater *in vitro* activity against NG than did penicillin, ciprofloxacin, azithromycin, and tetracycline. CPT was less active against NG than cefixime and ceftriaxone. These results suggest that CPT has the potential for use in treating NG infections.

Table: Activity Against *N. gonorrhoeae* isolates*

Antibiotic	N	MIC50	MIC90	Range		
Penicillin	403	0.5	4	0.008	-	32
Ciprofloxacin	403	0.008	16	0.002	-	32
Azithromycin	403	0.25	1	0.03	-	8
Tetracycline	404	1	4	0.06	-	32
Cefixime	219	0.03	0.03	0.002	-	0.125
Ceftriaxone	404	0.008	0.03	0.001	-	1
Ceftaroline	404	0.125	0.5	0.002	-	2

C1-182

Co-Opting the Cell Wall in Fighting Methicillin-Resistant *Staphylococcus aureus* (MRSA): Potent Inhibition of Penicillin-Binding Protein 2a (PBP 2a) by Ceftaroline

A. VILLEGAS-ESTRADA, M. LEE, D. HESEK, S. B. VAKULENKO, S. MOBASHERY;
Univ. of Notre Dame, Notre Dame, IN.

Background: Methicillin resistance in MRSA is due to the gene *mecA*, which encodes PBP 2a, a transpeptidase that plays a key role in cell wall synthesis. PBP 2a is not inhibited by most β -lactam antibiotics because of a closed conformation of its active site, which opens only in the course of catalytic function. Ceftaroline (CPT) is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including MRSA and multidrug-resistant *Streptococcus pneumoniae*, as well as common gram-negative pathogens. In this study, we characterize the mechanism of action of CPT with respect to PBP 2a inhibition.

Methods: MICs were determined by CLSI microdilution assay. The apparent first-order rate constant for acylation of PBP 2a by CPT was determined in the presence and absence of a cell wall surrogate. The deacylation rate constant (k_3) and reversible inhibition of PBP 2a in the presence CPT were measured.

Results: CPT is a potent inhibitor of PBP 2a with K_i of 330 ± 40 nM. The second-order rate constant for the encounter between PBP 2a and CPT (k_2/K_S) is $(2.4 \pm 0.1) \cdot 10^4 \text{ M}^{-1}\text{s}^{-1}$, indicating highly efficient formation of the inhibitory acyl-enzyme intermediate. In contrast, this process is very slow and inefficient for other β -lactam antibiotics, such as nitrocefin and imipenem ($<20 \text{ M}^{-1}\text{s}^{-1}$). PBP 2a inhibition was further enhanced in the presence of a cell wall surrogate, which binds in a saturable manner to an allosteric site in PBP 2a.

Conclusions: This study demonstrates that CPT effectively inhibits PBP 2a, a process that is enhanced in the presence of a cell wall surrogate. In the course of bacterial growth the occupancy of the allosteric site for the cell wall may be co-opted by CPT, and under these conditions the second-order rate constant approaches the clinically useful value of 10^4 - $10^5 \text{ M}^{-1}\text{s}^{-1}$. These findings help explain the anti-MRSA activity of CPT which is in contrast to marketed β -lactam antibiotics.

C1-183

Binding of Ceftaroline (CPT) to Penicillin-Binding Proteins (PBPs) of *Streptococcus pneumoniae* (SPN) and Methicillin-Resistant *Staphylococcus aureus* (MRSA)

H. MOISAN, M. PRUNEAU, F. MALOUIN;
Univ. de Sherbrooke, Sherbrooke, Canada.

Background: Emergence of multi-drug resistant MRSA and SPN has prompted development of new antibiotics. CPT is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including MRSA and multidrug-resistant SPN, as well as common gram-negative pathogens. CPT is currently in phase 3 development. We studied the affinity of CPT to PBPs of methicillin-susceptible *S. aureus* (MSSA), MRSA and SPN with varying susceptibility to penicillin.

Methods: PBPs were labeled using fluorescent Bocillin FL (BoFL). The binding affinity of β -lactams for PBPs was measured by adding increasing concentrations of the test drugs to membrane preparations (*S. aureus*) or whole cells (SPN) prior to addition of BoFL. For MRSA PBP2a, the samples were first exposed to clavulanate to saturate high affinity PBPs before adding the test drugs. The concentration of the drugs needed to block 50% of the binding of BoFL to each PBP (IC_{50}) was estimated by quantification of fluorescence after gel electrophoresis.

Results: Potency of the β -lactams against MRSA 67-0 was correlated with their affinity for PBP2a (CPT MIC 0.5 μ g/ml, IC_{50} 0.16 μ g/ml; vs oxacillin [OXA] and ceftriaxone [CRO] MIC >64 μ g/ml, IC_{50} >400 μ g/ml). CPT bound equally well to MSSA PBPs 1 to 3 (IC_{50} 0.03-0.1 μ g/ml), whereas OXA and CRO showed lower affinity for PBPs 2 and 3, respectively (IC_{50} £0.7 μ g/ml). CPT and CRO (MIC 0.008 and 0.03 μ g/ml, respectively) had comparable affinity for PBPs of SPN strain R6 with IC_{50} as low as 0.01 μ g/ml. Higher potency of CPT against SPN strain 2039 (MIC 0.12 μ g/ml) correlated best with its affinity to PBP2x (IC_{50} 0.2 μ g/ml) while CRO showed a higher MIC and PBP2x IC_{50} (1 and 0.6 μ g/ml, respectively).

Conclusion: The high affinity of CPT for MRSA PBP2a and PBP2x of SPN with a reduced susceptibility to PEN reflects its strong antibacterial activity against these pathogens, supporting the potential effectiveness of CPT in the treatment of MRSA and SPN infections.

C1-185

Spontaneous Mutation Frequency and Serial Passage Resistance Development Studies with Ceftaroline (CPT)

R. R. HINSHAW¹, R. D. SCHAADT¹, B. MURRAY¹, D. STAPERT¹, D. BIEK², Y. GE², G. E. ZURENKO¹, D. SHINABARGER¹;

¹Micromyx, Kalamazoo, MI, ²Cerexa, Inc., Alameda, CA.

Background: CPT is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common gram-negative pathogens. This study evaluated the potential for CPT resistance development in vitro for gram-positive and -negative pathogens.

Methods: The spontaneous mutation frequency of CPT for 11 different gram-positive and -negative pathogens was determined by inoculating agar containing CPT at multiples of the MIC. Resistance development during serial exposure was assessed using a microbroth dilution method used in MIC testing, by transferring cells from wells showing growth to fresh media for a total of 10 serial passages.

Results: Methicillin-susceptible *S. aureus* (MSSA), MRSA, community-acquired MRSA, vancomycin-intermediate *S. aureus* (VISA), penicillin-susceptible *S. pneumoniae* (PSSP), penicillin-resistant *S. pneumoniae* (PRSP), and β -lactamase negative *H. influenzae* did not develop detectable resistance to CPT using either method. Vancomycin-susceptible *Enterococcus faecalis* (VSE) had a spontaneous mutation rate of 1.25×10^7 , and the MIC was 4-fold higher after serial passage. Spontaneous CPT resistance was not detected for vancomycin-resistant *E. faecalis* (VRE), but a 4-fold increase in MIC developed during serial passage.

Conclusions: Resistance development to CPT was low or non-existent for the majority of the pathogens tested. The exceptions were VSE and VRE, which slowly developed resistance during serial passage, and VSE which exhibited detectable spontaneous resistance. Overall, CPT demonstrated a low propensity for resistance development for the pathogens tested.

C1-195

Capability of PZ-601 (SMP-601) to Select for Resistant Mutants of Vancomycin-Susceptible and Non-Susceptible MRSA

C. CLARK, K. KOSOWSKA-SHICK, P. MCGHEE, P. C. APPELBAUM;
Hershey Med. Ctr., Hershey, PA.

Background: PZ-601 is a carbapenem active against a range of Gram-positive and negative bacteria, including MRSA. We used multistep selection to compare the abilities of PZ-601, vancomycin and linezolid to select for resistant mutants against a range of methicillin-susceptible *S. aureus* (MSSA) and -resistant *S. aureus* (MRSA) strains with varying vanco resistance phenotypes.

Methods: In total, 2 MSSA and 8 MRSA isolates were tested (including 2 h VISA, 2 VISA, 2 VRSA); 9 of the 10 strains were recent Hershey isolates. CLSI macrodilution was used for MIC. Serial passages were done daily in MHB for each strain in subinhibitory drug concentrations, taking for each subsequent passage an inoculum from the tube one to two dilutions below the MIC that matched the turbidity of a growth control tube. Daily passages were continued until a >4-fold increase in MIC was found (minimum 14, maximum 50 passages). Resistant clones were subcultured ten times in drug-free medium to test stability of selected resistance and their identity genetically confirmed.

Results: Parental MICs ($\mu\text{g/ml}$) were as follows: PZ-601, 0.016-4; vanco, 2->64; linez, 2-4. PZ-601 yielded no resistant clones (as defined above) after 15 days. After 20 days daily subculture PZ-601 only yielded clones with MICs >4 $\mu\text{g/ml}$ (preliminary breakpoint) in one strain (8 $\mu\text{g/ml}$) whose parent MIC was 4 $\mu\text{g/ml}$ (stable after 10 drug-free subcultures). After 18-49 days in 6/10 strains the MICs increased from 0.016-2 $\mu\text{g/ml}$ (parents) to 0.125-8 $\mu\text{g/ml}$ (R clones). Linez yielded resistant clones after 15-48 days in 4/10 strains with MICs rising to 16-32 $\mu\text{g/ml}$ (R clones). Vanco produced no resistant clones in the 8 sensitive and intermediate strains tested.

Conclusions: PZ-601 has confirmed activity vs MRSA at a breakpoint of $\leq 4 \mu\text{g/ml}$ with a low propensity to select mutations after 20 days subculture.

C1-3719

In Vitro Activity and Aminoglycoside Synergy of Ceftaroline (CPT) Against Clinical Isolates of Hospital-Acquired (HA) Methicillin-Resistant *Staphylococcus aureus* (MRSA)

C. VIDAILLAC, S. N. LEONARD, M. J. RYBAK;
Wayne State Univ., Detroit, MI.

Background: Ceftaroline is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common gram-negative pathogens. CPT is currently in phase 3 development. CPT *in vitro* activity and its potential for synergy with tobramycin (TM) was evaluated in comparison to that of vancomycin (VM) against HA-MRSA isolates, including heterogeneous Vancomycin-Intermediate *S. aureus* (*hVISA*).

Methods: MICs and MBCs of CPT, VM, daptomycin, and linezolid were determined according to CLSI guidelines, for 200 clinical, molecularly-defined HA-MRSA isolates. Four randomly selected strains (including 1 *hVISA* and 1 *VISA*) were subsequently evaluated in time-kill experiments (TK) with CPT and VM alone or combined with TM, at $\frac{1}{4}$ MIC and $\frac{1}{2}$ MIC. Bactericidal activity was defined as ≥ 3 log kill and synergy, additive effect, antagonism and indifference were defined as > 2 log kill, < 2 but > 1 log kill, > 1 log growth and ± 1 log kill, respectively.

Results: CPT and VM MIC₅₀ and MIC₉₀ were 1 mg/L with a range of 0.25-2 mg/L and 1 and 2 mg/L with range of 0.25-4 mg/L, respectively. CPT MBC values were similar or 1-fold higher than the MIC. In TK experiments, CPT and TM alone at $\frac{1}{2}$ the MIC were cidal versus *hVISA* but not against *VISA* or MRSA. In contrast, VM alone was not cidal at either $\frac{1}{4}$ or $\frac{1}{2}$ MIC against MRSA, *hVISA* or *VISA*. Combination of CPT + TM was synergistic at $\frac{1}{2}$ MIC vs. MRSA but not *hVISA* or *VISA*. VM + TM only demonstrated synergy at $\frac{1}{2}$ MIC versus *hVISA*. No synergy was noted for CPT or VM at $\frac{1}{4}$ MIC against tested isolates.

Conclusions: At $\frac{1}{2}$ MIC, CPT alone or combined with TM exhibited greater activity than VM against tested HA-MRSA, including *hVISA* strains. Thus, the potential of increased activity of CPT in combination with aminoglycosides should be further explored both *in vitro* and *in vivo*.

C2-255

In Vitro Activity of Ceftaroline Against Penicillin-Resistant *Streptococcus pneumoniae* with Higher Amoxicillin vs. Penicillin MIC

A. FENOLL¹, L. AGUILAR², O. ROBLEDO¹, M. J. GIMÉNEZ², J. J. GRANZIO³, **D. BIEK**⁴, D. TARRAGÓ¹;
¹Inst. Salud Carlos III (ISCIII), Madrid, Spain, ²Sch. of Med., Univ. Complutense, Madrid, Spain, ³Granadatos SL, Madrid, Spain, ⁴Cerexa, Inc., Alameda, CA.

Background: Ceftaroline (CPT) is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin -resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *S. pneumoniae* (MDRSP), as well as common gram-negative pathogens. CPT is currently in phase 3 development. Penicillin (PEN)-resistant isolates of *S. pneumoniae* with higher amoxicillin (AMX) MIC vs. PEN MIC are challenging to treat because of their multidrug resistant phenotype. In this study, CPT was evaluated against these resistant isolates.

Methods: Susceptibility of 220 PEN-resistant (MIC³ 2 µg/ml) *S. pneumoniae* isolates with higher AMX MIC vs. PEN MIC were collected in the ISCIII during 2005-2007. Susceptibility of the isolates to CPT and comparators was determined by CLSI agar dilution assay. Cefuroxime sodium (CXM), cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP) and CPT were tested. CLSI M100-S17 susceptibility breakpoints were considered.

Results: MIC₅₀ (µg/ml), MIC₉₀ (µg/ml) and % susceptibility to study drugs for serotypes with >25 isolates with this resistance phenotype are shown in Table 1.

Conclusion: CPT exhibited the highest intrinsic activity against *S. pneumoniae* with MIC₉₀ values at least 2-doubling dilutions lower than for CRO and other beta-lactams tested. These in vitro data suggest that CPT has strong clinical potential for the treatment of infections caused by resistant pneumococci in community- or hospital-acquired pneumonia.

	Serotype 9V (n=69)		Serotype 14 (n=65)		Serotype 6B (n=33)		Serotype 19A (n=27)	
	MIC50/90	%S	MIC50/90	%S	MIC50/90	%S	MIC50/90	%S
PEN	2/4	0.0	2/4	0.0	2/2	0.0	2/4	0.0
AMX	8/16	0.0	8/16	0.0	8/8	0.0	8/8	0.0
CXM	8/8	0.0	8/16	0.0	8/16	0.0	8/16	0.0
CTX	1/2	82.6	2/2	26.2	1/2	66.7	2/4	33.3
CRO	0.5/1	98.6	1/1	93.8	1/1	100	1/2	88.9
FEP	2/4	18.8	2/4	15.4	2/4	27.3	2/2	18.5
CPT	0.12/0.12	-	0.12/0.25	-	0.12/0.25	-	0.12/0.25	-

C2-1974

Antimicrobial Activity of Ceftriaxone (CPT) Tested Against Contemporary (2008) Bacteria Isolated from Community-Acquired Respiratory Tract Infections (CARTI), Including Oxacillin-Resistant *S. aureus* (MRSA)

H. S. SADER, T. R. FRITSCHKE, R. N. JONES; JMI Lab., North Liberty, IA.

Background: CPT is a new N-phosphono prodrug of an anti-MRSA cephalosporin that has high affinity for PBP 2a and demonstrated activity against MRSA and other pathogens responsible for CARTI. CPT, currently in phase III clinical development, was evaluated for potency against CARTI pathogens.

Methods: Isolates were consecutively collected from CARTI in hospitals from the USA (26) and Europe (EU; 28). Susceptibility (S) was tested by CLSI broth microdilution methods against CPT and antimicrobials used to treat CARTI. *S. aureus* isolates were obtained from patients with pneumonia less than 72 hrs after hospitalization.

Results: CPT ($MIC_{50/90} \leq 0.008/0.12 \mu\text{g/ml}$) was 8-fold more potent than ceftriaxone (CRO; $MIC_{50/90} \leq 0.25/1 \mu\text{g/ml}$) and 64-fold more potent than cefuroxime ($MIC_{50/90} \leq 1/8 \mu\text{g/ml}$) against *S. pneumoniae* (SPN). Penicillin resistance (R) was high among SPN; only 59.9 and 84.6% strains were inhibited at ≤ 0.06 and $\leq 2 \mu\text{g/ml}$, respectively, while amox/clav inhibited 80.1% at $\leq 2 \mu\text{g/ml}$. SPN R was also high for azithromycin (38.0%), clindamycin (20.2%), co-trimoxazole (26.2%) and tetracycline (22.3%). CPT was very active against *H. influenzae* ($MIC_{90}, 0.015 \mu\text{g/ml}$, including β -lactamase(+) strains (22.6%), and 16-fold more potent than CRO against oxacillin-S *S. aureus* (MSSA). The highest CPT MIC value among MRSA was $2 \mu\text{g/ml}$, and 95.8% of isolates were inhibited at $\leq 1 \mu\text{g/ml}$ of CPT.

Conclusion: CPT exhibited high activity against bacterial pathogens from CARTI recently (2008) collected in USA and EU medical centers, including CA-MRSA and other R strains. Based on these results, CPT appears to be a promising agent for the therapy of CARTI.

Organism (no.)	Cumulative % inhibited at MIC (mg/ml) of:								
	≤ 0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2
<i>S. pneumoniae</i> (287)	55.1	65.9	69.3	77.4	92.0	99.3	100.0	-	-
<i>H. influenzae</i> (177)	82.5	95.5	96.1	99.4	100.0	-	-	-	-
MSSA (30)	0.0	0.0	0.0	0.0	9.1	100.0	-	-	-
MRSA (24)	0.0	0.0	0.0	0.0	0.0	0.0	45.8	95.8	100.0

C2-3930

In Vitro Activity of SMP-601 (PZ-601) Against Anaerobic Bacteria

K. TANAKA¹, H. MIKAMO², T. GOTO¹, K. WATANEBE¹;

¹Div. of Anaerobe Res., Life Sci. Res. Ctr., Gifu Univ., Gifu, Japan, ²Dept. of Infection Control and Prevention, Aichi Med. Univ., Aichi, Japan.

Background: SMP-601 is a new parenteral carbapenem with broad antimicrobial spectrum. It showed potent activity against various aerobic bacteria, including methicillin-resistant *Staphylococcus*, penicillin-resistant *S. pneumoniae*, and ESBL-producing bacteria. SMP-601 showed potent activity against *B. fragilis*, while there have been no reports on anti-anaerobic activity of SMP-601 against other species. We evaluated the *in vitro* anti-anaerobic activity of SMP-601 against various reference strains and clinical isolates.

Methods: The anti-anaerobic activity of SMP-601 was compared with other carbapenems (panipenem, meropenem and doripenem), clindamycin and metronidazole. A total of 70 Gram (+) and Gram (-) reference strains of anaerobes and some fastidious microaerophilic species (63 species, 26 genera) were examined. Clinical isolates of *B. fragilis* group (66 strains), *Prevotella* (48 strains), *Fusobacterium* (24 strains), *C.difficile* (19 strains), anaerobic Gram (+) cocci (94 strains) and other Gram (+) and Gram (-) strains (74 strains) were also studied. MICs were determined by the CLSI agar dilution method.

Results: SMP-601 showed strong activity against anaerobes, compared with other carbapenems. SMP-601 inhibited most of the reference strains at 2 µg/mL. Among the clinical isolates, SMP-601 showed strong activity, compared with other carbapenems against non-*fragilis* *Bacteroides*, *Prevotella*, *Fusobacterium*, and anaerobic Gram (+) cocci, with MIC₉₀s of 2, 0.06, 0.5, and 0.03-1 µg/mL, respectively. SMP-601 was the most potent agent against *C. difficile* among the carbapenems investigated, resulting in MIC₉₀ of 0.5 µg/mL.

Conclusions: SMP-601 showed broad spectrum against Gram (+) and Gram (-) anaerobic bacteria, compared with other carbapenems investigated. Since SMP-601 has longer half-life compared with other launched carbapenems, SMP-601 would be one of superior carbapenems against various anaerobic infections.

D-291b

Use of NXL104, a β -Lactamase Inhibitor, to Detect *Klebsiella pneumoniae* Carbapenemase (KPC) in *Enterobacteriaceae*

P. LEVASSEUR¹, C. MIOSSEC¹, A. GIRARD¹, D. SHLAES²;

¹Novoxel SA, Romainville, France, ²Anti-Infectives Consulting, Stonington, CT.

Background: NXL104 is a non- β -lactam covalent inhibitor of a broad spectrum of serine β -lactamases of classes A and C, including ESBLs (Extended Spectrum β -Lactamases) and KPC carbapenemases. The KPCs are of greater concern, conferring resistance to all β -lactams including the currently marketed inhibitors and the carbapenems. KPC enzymes have been reported in a variety of *Enterobacteriaceae* but remain difficult for clinical microbiology laboratories to detect. Ceftazidime (CAZ) + NXL104 disks are being developed to support the clinical development of CAZ/NXL104. We have invented a novel use for NXL104 as a diagnostic reagent for the detection of KPC β -lactamases expressed by strains of *Enterobacteriaceae* species.

Methods: Bacterial isolates tested, six clinical isolates known to harbour KPC-2 or KPC-3 (3 *K. pneumoniae*, 1 *Escherichia coli* and 2 *Enterobacter cloacae*) and five clinical *Enterobacteriaceae* isolates without KPCs but with ESBLs, including the *K. pneumoniae* ATCC 700603 (SHV-18). MICs were determined as per CLSI microbroth dilution technique with ceftazidime (CAZ) and imipenem (IPM) alone or combined with NXL104 at a fixed concentration of 4 μ g/mL. Double disk synergy test for detection of KPC: Mueller-Hinton agar plates were inoculated with a lawn of $\sim 10^8$ CFU/mL adjusted test strains. A 10 μ g IPM disk was placed at a distance of ~ 20 -22 mm apart from a disk of 30 μ g CAZ+60 μ g NXL104. Plates were inverted and incubated at 37°C overnight.

Results: Synergy, indicating the presence of carbapenemase, was seen as an expansion of the IPM zone adjacent to the CAZ/NXL104 disk only for strains known to carry a KPC enzyme. These results correlated with the decrease in IPM MICs in the presence of NXL104 (0.125 - 2 μ g/mL) compared to its absence (8 - 64 μ g/mL).

Conclusion: These results demonstrate CAZ/NXL104 double disk synergy test as a potentially useful method for detecting KPCs in *Enterobacteriaceae* species in clinical microbiology laboratories.

D-2228

An Evaluation of the Sensititre® MIC Susceptibility System Compared to the CLSI (M31) Reference Broth Microdilution Method with the New Antimicrobial Agent, Cefovecin

C. BASTULLI¹, N. HOLLIDAY¹, C. KNAPP¹, S. KILLIAN¹, C. LINDEMAN²;

¹TREK Diagnostic Systems, Cleveland, OH, ²Pfizer Animal Hlth., Kalamazoo, MI.

Background: Cefovecin (FOV) (Pfizer Animal Health, Kalamazoo, MI), is a new semi-synthetic extended-spectrum cephalosporin antibiotic. It is indicated for treatment of bacterial infections in small animals. An evaluation was undertaken to determine the accuracy and reproducibility of the Sensititre 18 - 24 hour dried susceptibility system (manufactured by TREK Diagnostic Systems, Cleveland, OH) with Cefovecin, compared to the CLSI (M31) reference broth microdilution method (BMD). The range tested for Cefovecin was (0.0005 - 32 µg/ml) on both the Sensititre and BMD plates.

Methods: Two hundred isolates (20 *Staphylococcus intermedius*, 20 *Staphylococcus aureus*, 20 *Streptococcus canis* (group G), 20 *Escherichia coli*, 20 *Proteus mirabilis*, 20 *Pasteurella multocida*, 20 *Haemophilus somnus*, 20 *Actinobacillus pleuropneumoniae*, 20 *Bacteroides fragilis* and 20 *Prevotella* species) were tested with FOV and 3 comparators, Cefpodoxime, Ceftiofur, and Cefoxitin, using the Sensititre 18-24 h dried susceptibility plate and by CLSI (M31) BMD for comparison. For reproducibility testing, 10 isolates were tested 3 times daily over a period of three days. Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected quality control ranges for FOV and all comparators.

Results: Comparison of the Sensititre plate to the CLSI (M31) BMD resulted in 100% essential agreement (+/- one log₂ dilution) for FOV and 100% for comparators. Reproducibility was calculated as the percentage of results within +/- one log₂ dilution of the modal value. Overall agreement for the reproducibility of FOV and for comparators was 100%.

Conclusions: This evaluation indicates that the Sensititre 18-24 hour dried susceptibility system with FOV is equivalent to the CLSI (M31) BMD and is an acceptable method for susceptibility testing of FOV.

D-2232

Effects of In Vitro Test Method Variables on Ceftaroline Activity Against Aerobic Gram-Positive and -Negative Pathogens

D. M. CITRON, Y. A. WARREN, K. L. TYRRELL, E. J. C. GOLDSTEIN;
R.M. Alden Res. Lab., Culver City, CA.

Background: Ceftaroline (CPT) is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common gram-negative pathogens. CPT is currently in phase 3 development. This study determined the effects of different *in vitro* test conditions on the MICs of 30 strains representing 10 species of clinically important organisms.

Methods: The strains were clinical or ATCC quality control (QC) strains of *E. coli*, *K. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, *E. faecalis*, *S. pyogenes*, and *S. pneumoniae*. The CLSI broth microdilution method in cation adjusted Mueller Hinton broth was used with the following respective modifications: 50 mg/L Ca⁺⁺, 5% NaCl, pH 6, pH 8, inoculum at 10⁴ cfu/ml and 10⁶ cfu/ml, 10% and 50% human serum, 2.5% laked horse blood (LHB), HTM broth, incubation in 5% CO₂ and incubation anaerobically. In addition, agar dilution MICs in plain MHA, MHA with LHB, and HTM with 1.5% agar were determined.

Results: MICs for the QC strains were within their acceptable ranges. 5% NaCl inhibited growth and/or reduced MICs for *E. coli* and *K. pneumoniae* and completely inhibited growth of *M. catarrhalis*, and *H. influenzae* and all streptococci. The 10⁶ cfu/ml inoculum increased the MIC five-fold for 1 of 3 *E. coli* and 1 of 3 *K. pneumoniae* strains. The higher inoculum also increased MICs 3 to 5 fold for *M. catarrhalis*, while the addition of blood or serum enhanced their growth without changing the MIC. All other variables had minimal effect and the MICs were generally within one dilution of the reference method.

Conclusions: The *in vitro* antibacterial activity of CPT was not adversely affected by most modifications in testing methods.

D-2249

Comparative Evaluation of Ceftaroline MIC Testing with Etest and CLSI Broth Microdilution Methods

A. ENGELHARDT, A. YUSOF, P. HO, K. SJÖSTRÖM, C. JOHANSSON; AB BIODISK, Solna, Sweden.

Background: Ceftaroline (CPT) is a novel, parenteral, broad-spectrum cephalosporin with bactericidal activity against Gram-positive organisms, including methicillin-resistant (MR) *S. aureus* (MRSA), multidrug-resistant *S. pneumoniae*, as well as common Gram-negative pathogens. CPT is currently in phase 3 development. The performance of a new Etest CPT strip was compared to the CLSI broth microdilution (BMD) method using a set of challenge strains.

Methods: Etest CPT (MIC 0.002 - 32 µg/mL) and BMD were performed with recommended procedures. Test strains (39) used are listed in the result table. The following ATCC® strains were tested: *E. coli* 25922, *P. aeruginosa* 27853, *E. faecalis* 29212, *S. aureus* 29213 (methicillin susceptible *S. aureus*, MSSA), 43300 (MRSA), 700699 (vancomycin intermediate *S. aureus*, VISA) and 700698 (hVISA). The effects of inoculum variation (100 fold) were studied with QC strains.

Results: Excellent agreement was seen between Etest and BMD (100% ±1 dilution) for the challenge organisms that had a wide range of MIC values. QC results for both methods fell within the CLSI specifications. Inoculum effects on MIC values were minimal.

Conclusions: Substantially equivalent MIC results for CPT can be obtained by Etest and the CLSI reference method. The wide concentration range in Etest (15 dilutions) and simplicity of use makes it a useful and convenient tool for MIC testing during drug development and for subsequent clinical use.

Species (N)	MIC range µg/mL
<i>E. coli</i> (6)	0.047-1
<i>Enterobacter</i> spp. (7)	0.047- >32
<i>K. pneumoniae</i> (6)	0.094-12
<i>P. mirabilis</i> (2)	0.047-0.094
<i>Serratia</i> & <i>Citrobacter</i> spp.(2)	0.75-1
<i>E. faecalis</i> (2)	0.5-2
MSSA (5)	0.094-0.25
MRSA+VISA/hVISA (6)	0.38-1.5
MR-coag. neg. staphylococci (3)	0.094-0.38

F1-344

In Vitro Antibacterial Activity of Sulopenem: A New Oral Penem Antimicrobial Versus Recent Bacterial Clinical Isolates

M. D. HUBAND¹, T. D. GOOTZ ¹, M. A. COHEN ², L. M. MULLINS ¹, S. P. MCCURDY ¹, L. A. BRENNAN ¹, J. M. DUIGNAN ¹, P. J. PAGANO ², R. W. MURRAY ²; ¹Pfizer Global R&D, Groton, CT, ²Pfizer Global R&D, Ann Arbor, MI.

Background: The continuing emergence of antimicrobial resistance in bacterial species (multi-drug resistant *S. pneumoniae*, ESBL+ *Enterobacteriaceae*), has created a need for new antibacterial compounds. Sulopenem is an orally active antibacterial being developed to treat infections caused by susceptible and multi-drug resistant organisms. This study examined the antibacterial activity of sulopenem and comparator compounds against a collection of geographically diverse recent bacterial clinical isolates.

Methods: Microbroth dilution MICs (expressed in µg/mL) and their interpretation followed CLSI guidelines. In vitro time-kill studies were also performed.

Results: see table

Conclusions: MIC and time-kill data support further consideration of sulopenem as a candidate for the treatment susceptible and multi-drug resistant infections, including those caused by penicillin-resistant *S. pneumoniae* and ESBL producing *Enterobacteriaceae*.

* Includes 24 PSSP, 29 PISP, 36 PRSP, and 25 levofloxacin-R PRSP strains
Sulopenem MIC₉₀s ranged 0.03-1 µg/mL versus the clinically significant bacterial pathogens tested. This high in vitro potency was confirmed by in vitro time-kill studies.

Organisms (# Tested)	Sulopenem MICs (µg/mL)		
	Range	MIC ₅₀	MIC ₉₀
<i>Staphylococcus aureus</i> MSSA(19)	0.06-0.125	0.06	0.125
<i>Streptococcus pneumoniae</i> * (114)	0.008-1	0.06	0.5
<i>S. pyogenes</i> (28)	0.015-0.25	0.03	0.03
<i>S. agalactiae</i> (21)	0.03-0.25	0.06	0.125
<i>Listeria monocytogenes</i> (10)	0.06-0.125	0.06	0.125
<i>Enterobacteriaceae</i> (200)	0.015-64	0.125	1
<i>E. coli</i> ESBL+ (17)	0.015-0.25	0.03	0.125
<i>K. pneumoniae</i> ESBL+ (15)	0.03-0.5	0.06	0.25
<i>Moraxella catarrhalis</i> (30)	0.008-0.25	0.015	0.25
<i>Haemophilus influenzae</i> (65)	0.06-1	0.125	0.5

F1-343

Real-Time Evaluation of a New Carbapenem, ME1036, in a Rat *Staphylococcus aureus* Endocarditis (IE) Model Using In Vivo Bioluminescent Imaging

Y. Q. XIONG^{1,2}, Y. LI¹, W. ABDEL HADY¹, D. BIEK³, Y. GE³, A. S. BAYER^{1,2};

¹LABioMed at Harbor-UCLA, Torrance, CA, ²Geffen Sch. of Med. at UCLA, Los Angeles, CA, ³Cerexa, Inc, Alameda, CA.

Background: ME1036 is a new parenteral carbapenem with activity against a variety of resistant gram-positive and -negative pathogens, including methicillin-susceptible and -resistant *S. aureus* (MSSA and MRSA) and extended spectrum β -lactamase (ESBL)-producing Enterobacteriaceae. In this study, we tested the efficacy of ME1036, vancomycin (VAN) and daptomycin (DAP) in real-time in a rat IE model due to a bioluminescently engineered, biofilm-positive MSSA strain.

Methods: IE was induced following transcarotid-transaortic valve indwelling catheterization. At 3 days after infection, rats received either: i) no therapy (control); ii) ME1036 100 mg/kg + Cilastatin (an inhibitor of renal dehydropeptidase) 50 mg/kg, iv, bid; iii) VAN 120 mg/kg, subcutaneous (SC), bid; or iv) DAP 10 mg/kg, SC, once daily. Cardiac bioluminescence signals (BLS) were quantified daily using a highly sensitive *in vivo* imaging system (IVIS®). 24 hours after the last antibiotic dose, half of the rats were sacrificed and target tissues were quantitatively cultured. Remaining animals were left treatment-free for 3 more days to assess relapse.

Results: There were significant correlations between cardiac BLS and *S. aureus* densities in vegetations in all treatment groups ($r^2=0.903$). ME1036 and VAN significantly decreased *S. aureus* densities vs. controls. ME1036 also significantly prevented relapse (Table).

Conclusions: ME1036 had excellent efficacy and prevented relapse vs. VAN and DAP in this IE model of severe *S. aureus* infection.

Group (# of rats)		Log ₁₀ CFU/g. tissue \pm SD		
		Vegetations	Kidneys	Spleen
Control (6)		9.76 \pm 0.72	7.80 \pm 0.47	6.94 \pm 0.84
ME1036+Cilastatin (7)		6.07 \pm 0.77*	4.18 \pm 0.45*	3.68 \pm 0.71*
Vancomycin (7)	TREATMENT	6.76 \pm 0.98*	4.15 \pm 1.20*	4.28 \pm 0.89*
Daptomycin (6)		7.64 \pm 0.32	5.53 \pm 0.57	5.49 \pm 0.38
ME1036+Cilastatin (8)		5.17 \pm 1.51**	3.70 \pm 1.22**	3.59 \pm 1.04**
Vancomycin (7)	RELAPSE	8.15 \pm 1.09	6.21 \pm 0.41	6.34 \pm 0.53
Daptomycin (6)		8.36 \pm 1.38	6.99 \pm 1.06	7.02 \pm 1.15

* $P < 0.05$ vs. controls;

** $P < 0.05$ vs. VAN and DAP relapses.

F1-345

Antipneumococcal Activity of Sulopenem Compared to Other Agents by MIC Methodology

L. M. EDNIE, P. C. APPELBAUM; Hershey Med. Ctr., Hershey, PA.

Background: Amongst currently available oral β -lactams, amoxicillin has the greatest potency against pneumococci with raised penicillin G MICs. Parenteral β -lactams such as ceftriaxone, imipenem and meropenem are also very potent against these strains.

Purpose: Sulopenem is a penem antimicrobial in current development. This study examined the activity of amoxicillin, amoxicillin/clavulanate, sulopenem, imipenem, meropenem, ceftriaxone, cefuroxime, cefpodoxime, cefdinir, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, azithromycin and clarithromycin.

Methods: Agar dilution (5% sheep blood plates) incubated in air with plates incubated overnight at 35°C was used. We tested 80 penicillin susceptible (MICs <0.125 μ g/ml) , 88 penicillin intermediate (MICs 0.125-1 μ g/ml) and 129 penicillin resistant (MICs >1 μ g/ml) pneumococci: of these, 152 were macrolide resistant and 30 quinolone resistant (both groups with defined genotypes).

Results: MIC₅₀ and MIC₉₀ values (μ g/ml) see table. Sulopenem was very active against all pneumococci irrespective of resistance phenotype, with MICs ranging between \leq 0.004 and 1.0 μ g/ml for all strains and potency very similar to those of imipenem and meropenem. β -Lactam and macrolide MICs rose with those of penicillin G. Other oral cephalosporins had higher MICs than amoxicillin. Moxifloxacin had the lowest MICs of quinolones tested.

Conclusions: Sulopenem was very active against all pneumococci tested with MICs similar to those of imipenem and meropenem.

Drug	Penicillin S	Penicillin I	Penicillin R	Macrolide R	Quinolone R
Amox	0.03/0.03	0.25/1	2/8	0.25/8	0.06/4
Amox/clav	0.03/0.03	0.25/1	2/4	0.25/4	0.06/2
Sulop	0.008/0.016	0.06/0.25	0.25/0.5	0.125/0.5	0.03/0.5
Imip	0.008/0.008	0.03/0.125	0.25/0.25	0.06/0.25	0.016/0.25
Merop	0.016/0.016	0.06/0.25	0.5/0.5	0.125/0.5	0.03/0.5
Ceftriax	0.03/0.06	0.125/1	1/2	0.25/2	0.125/2
Cefurox	0.03/0.125	0.25/4	4/16	0.5/8	0.25/8
Cefpodox	0.03/0.06	0.25/2	4/8	0.25/4	0.25/8
Cefdinir	0.06/0.125	0.5/4	8/8	0.5/8	0.25/8
Cipro	2/32	1/2	2/8	1/2	32/>32
Levo	1/16	1/2	1/4	1/2	16/32
Gati	0.5/4	0.25/0.5	0.5/1	0.5/0.5	4/8
Moxi	0.25/4	0.125/0.25	0.25/0.5	0.125/0.25	2/4
Azithro	2/>64	2/>64	0.125/>64	>64/>64	0.125/>64
Clarithro	0.5/>64	1/>64	0.06/>64	64/>64	0.03/32

F1-346

Time-Kill Analysis of the Antipneumococcal Activity of Sulopenem, an Experimental Penem, Compared with Other Agents

L. M. EDNIE, P. C. APPELBAUM; Hershey Med. Ctr., Hershey, PA.

Background: Drug-resistant pneumococci have become a worldwide problem.

Objective: Sulopenem is a penem antibacterial under current development. We used time-kill to compare activities of sulopenem with amoxicillin, amoxicillin/clavulanate, imipenem, meropenem, ertapenem, ceftriaxone, cefuroxime, cefpodoxime, cefdinir, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, azithromycin, telithromycin against 2 pen S, 5 pen I and 5 pen R pneumococcal strains (including 6 macrolide R and 2 quinolone R strains).

Methods: Macrobroth dilution MICs were in cation-adjusted Mueller-Hinton broth + 5% lysed horse blood according to CLSI specifications. Time-kills tested viability in the same medium after 3 h, 6 h, 12 h and 24 h incubation in a shaking water bath in the presence of antibiotic.

Results: MICs ($\mu\text{g/ml}$) were as follows: sulop, 0.008-1.0; amox 0.03-8.0; amox/clav 0.03-8.0; imip 0.004-0.5; merop 0.008-2.0; ertap 0.03-4.0; ceftriax 0.016-4.0; cefurox 0.016-16.0; cefpodox 0.016-8.0; cefdinir 0.06-16.0; cipro 1->32.0; levo 1.0-16.0; gati 0.25-4.0, moxi 0.12-4.0; azithro 0.03->64.0; telithro 0.004-0.12. The numbers of strains showing 90% (-1), 99% (-2) and 99.9% (-3) killing at the MIC/2 x MIC were (**siehe nächste Folie**). Sulopenem at 2 x MIC was bactericidal against all 12 strains tested after 24 h and against 11/12 strains after 12 h. Other parenteral and oral β -lactams gave similar kill patterns relative to differing MICs. Quinolones were cidal against all strains tested at 2 x MIC after 24 h and macrolides gave slow killing.

Conclusions: Sulopenem gave MICs and kill kinetics similar to those of imipenem and meropenem against all strains tested.

F1-346 (Forts.)

Drug	3 h			6 h			12 h			24 h		
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3
Amox	11/11	4/5	0/0	12/12	7/9	3/5	12/12	12/12	10/11	11/12	11/12	10/12
Amox/clav	9/11	3/4	0/0	12/12	7/8	4/6	12/12	12/12	10/11	12/12	11/12	9/12
Sulopenem	10/10	5/5	0/1	12/12	8/9	3/4	12/12	12/12	11/11	12/12	11/12	10/12
Imipenem	12/12	4/5	1/2	12/12	8/9	3/4	12/12	12/12	10/11	12/12	11/12	10/12
Meropenem	10/11	3/3	0/0	12/12	6/9	2/2	12/12	12/12	10/10	12/12	10/12	10/12
Ertapenem	9/11	3/3	0/0	12/12	8/8	2/2	12/12	12/12	9/9	11/12	10/12	9/12
Ceftriaxone	7/10	2/2	0/0	11/12	5/8	2/3	12/12	11/12	6/10	11/12	8/12	8/12
Cefuroxime	8/9	3/3	0/0	11/11	6/7	1/2	12/12	12/12	7/9	9/12	7/12	6/12
Cefpodoxime	7/9	1/2	0/0	10/11	6/7	0/1	12/12	10/12	6/8	10/12	10/12	6/12
Cefdinir	8/8	1/1	0/0	11/12	6/7	1/2	12/12	12/12	5/9	11/12	10/12	7/12
Ciprofloxacin ^a	8/9	0/4	0/0	9/10	4/6	1/3	10/10	8/10	5/7	7/10	5/10	4/10
Levofloxacin ^a	9/10	2/4	0/0	10/10	5/8	1/2	10/10	10/10	5/9	8/10	8/10	7/10
Gatifloxacin ^a	5/10	1/2	0/0	8/10	4/8	1/1	10/10	8/10	4/7	9/10	8/10	7/10
Moxifloxacin ^a	4/9	0/2	0/0	9/10	2/4	1/2	9/10	8/10	2/5	8/10	6/10	5/10
Azithromycin ^b	1/1	0/0	0/0	3/3	1/1	1/1	5/8	3/5	1/3	8/8	5/8	2/8
Telithromycin	2/7	0/0	0/0	6/8	0/3	0/0	8/9	4/5	2/3	5/11	4/9	3/8

F1-347

Anti-Anaerobic Activity of Sulopenem Compared to Other Agents

L. M. EDNIE, P. C. APPELBAUM; Hershey Med. Ctr., Hershey, PA.

Background: β -Lactamase production is the rule amongst the *B. fragilis* group but is also encountered amongst other anaerobic Gram-negative bacilli.

Purpose: This study examines the activity of sulopenem, a penem under current development, compared with ampicillin, amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, imipenem, clindamycin and metronidazole against 101 *B. fragilis* group, 100 *Prevotella/Porphyromonas* spp, 60 fusobacteria, 50 anaerobic Gram-positive cocci, 60 anaerobic Gram-positive nonsporeforming rods and 60 clostridia.

Methods: CLSI agar dilution MICs were determined on enriched Brucella laked sheep blood agar with inocula of 10^5 cfu/spot. Clavulanate and sulbactam were combined with amoxicillin and ampicillin at 1:2 ratios and tazobactam with piperacillin at a fixed concentration of 4 μ g/ml.

Results: MIC₅₀s and MIC₉₀s (μ g/ml) were as follows (see table). Sulopenem was very active against all organism groups with the exception of lactobacilli, with MICs \leq 4 μ g/ml. Imipenem MICs were lower for lactobacilli but otherwise corresponded well with those of sulopenem. Some Gram-negative rods and Gram-positive cocci were clindamycin resistant and metronidazole was inactive against most Gram positive non-sporeforming rods.

Conclusions: Sulopenem was very active against clinically significant anaerobes with MICs similar to those of imipenem. Some Gram-negative organisms and clostridia were clindamycin resistant and metronidazole was inactive against most Gram-positive non-sporeforming rods.

Drug	<i>B. fragilis</i> <i>gp</i> (101)	<i>Prev</i> / <i>Porph</i> (100)	<i>Fusob</i> (60)	Anaer G+ cocci (50)	Anaer G + nonsp rods (60)	Clostridia (60)	All strains (431)
Amp	32/>128	2/64	1/ 2	\leq 0.125/1	0.25/1	0.25/2	1/64
Amox/clav	0.5/4	\leq 0.125/2	1/ 2	\leq 0.125/0.5	0.25/1	0.25/1	0.5/2
Amp/sulb	1/8	0.25/2	1/ 2	\leq 0.125/0.5	0.25/1	0.25/2	0.5/4
Pip/tazo	1/8	\leq 0.125/ \leq 0.125	0.25/4	\leq 0.125/0.5	1/16	0.5/8	0.25/8
Sulop	0.25/1	0.125/0.25	0.25/0.5	0.125/0.5	0.5/4	0.5/4	0.25/1
Imip	0.25/0.5	0.03/0.06	0.5/1	0.06/0.12	0.125/0.5	0.25/4	0.06/1
Clinda	1/>32	\leq 0.016/0.125	0.125/16	0.125/1	0.125/2	2/16	0.25/16
Metro	1/1	1/ 2	0.25/0.5	0.5/1	>16/>16	1/1	1/ 4

F1-348

Sulopenem (Sul): In vitro Potency, Spectrum of Activity, MIC and Disk Breakpoints

S. D. BROWN, M. M. TRACZEWSKI, The Clinical Microbiology Institute;
Clinical Microbiol. Inst., Inc, Wilsonville, OR.

Background: Sul is a new penem antibacterial with potent activity against gram-positive and gram-negative pathogens.

Methods: Using CLSI broth microdilution and disk diffusion methodology (10 µg disk), the present study was designed to: 1) assess the *in vitro* activity of Sul against 983 broad spectrum pathogenic bacterial isolates in comparison with other antimicrobials, and 2) recommend preliminary MIC and disk diffusion breakpoints.

Results: MIC_{90s} (µg/ml) of selected groups of isolates and drugs are as follows (see table 1):

Conclusions: Sul was very active against most strains of methicillin-susceptible staph, streptococci, *Enterobacteriaceae*, *P. multocida*, *L. monocytogenes*, *M. catarrhalis*, *H. influenzae*, and the majority of the anaerobes. The activity of Sul against strains of enterococci, *Pseudomonas spp.*, *C. jeikeium*, *S. maltophilia*, and methicillin-resistant staph was relatively low. The following MIC & disk diffusion breakpoints based on in vitro susceptibility profiles are proposed:

Selected Groups	Sul	Imipenem	Ertapenem	Groups	MIC Breakpoints (µg/ml) (S, I, R)	Disk Diffusion Breakpoints(mm) 10 µg Disk (S, I, R)
<i>S. aureus</i> (110)	1	0.25	2	Staphylococci	≤4, 8, ≥16	≥ 29, 26-28, ≤ 25 mm
Coag-Neg Staph (63)	1	0.25	4			
<i>Enterobacteriaceae</i> (116)	1	2	0.12	<i>Enterobacteriaceae</i> and Non- <i>Enterobacteriaceae</i>	≤ 4, 8, ≥ 16	≥ 21, 18-20, ≤ 17 mm
<i>L. monocytogenes</i> (22)	0.12	0.06	0.5			
Streptococci (161)	1	0.25	1	<i>S. pneumoniae</i> and α-strep.	≤ 1, 2, ≥ 4	N.A.
<i>S. pneumoniae</i> (81)	1	0.5	2			
<i>Haemophilus spp.</i> (91)	0.5	1	0.12	β-streptococci	≤ 0.12, 0.25, ≥ 0.5	N.A.
<i>M. catarrhalis</i> (22)	0.12	0.06	0.03			
All Anaerobes Combined (304)	2	4	2	<i>C. jeikeium</i> and <i>L. monocytogenes</i>	≤ 4, 8, ≥ 16	≥ 34, 31-33, £30 mm
				<i>H. influenzae</i>	≤ 1 with no I or R	≥ 23 mm with no I or R
				Anaerobes	≤ 4, 8, ≥ 16	N.A.

F1-349

Quality Control Parameters for Sulopenem Broth Microdilution Susceptibility Tests

S. D. BROWN, M. M. TRACZEWSKI, Clinical Microbiology Institute;
Clinical Microbiol. Inst., Inc, Wilsonville, OR.

Background: Sulopenem is a new penem antimicrobial with activity against a wide variety of microorganisms.

Methods: An eight-laboratory study was conducted to generate data to determine quality control (QC) limits for five standard QC organisms when testing susceptibility to sulopenem by the broth microdilution method. Three different lots of Mueller-Hinton broth were used. The broth was supplemented with 3% lysed horse blood for testing *S. pneumoniae* or made up as *Haemophilus* Test Medium for testing *H. influenzae*. All susceptibility tests were performed by methods outlined by the CLSI. Each laboratory performed 30 MIC determinations for each QC strain.

Results: Colony counts ranged from 7×10^4 to 2.2×10^6 . Significant lot-to-lot variation was not observed. All tests were very reproducible and the following quality control limits are proposed (see table):

Conclusions: Microbroth dilution quality control ranges are proposed for Sulopenem against 5 quality control strains recommended by the CLSI. These ranges have been proposed and accepted by the CLSI Subcommittee on Antimicrobial Susceptibility Testing.

<u>Organism (ATCC)</u>	MIC ($\mu\text{g/ml}$)	
	<u>Limits</u>	<u>% in Range</u>
<i>E. coli</i> (25922)	0.015 - 0.06	100%
<i>S. aureus</i> (29213)	0.015 - 0.12	100%
<i>E. faecalis</i> (29212)	2-8	98.3%
<i>S. pneumoniae</i> (49619)	0.03 - 0.12	100%
<i>H. influenzae</i> (49766)	0.06 - 0.25	100%

F1-350

Sulopenem: Effects of Environmental Variation on MICs and Short-Term Stability

M. M. TRACZEWSKI, S. D. BROWN, Clinical Microbiology Institute;
Clinical Microbiol. Inst., Inc, Wilsonville, OR.

Background: Sulopenem is an orally administered penem with potency against resistant gram-positive and gram-negative pathogens.

Methods: Using *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212 and CLSI broth microdilution (M7-A7) methodology, the present study was designed to: 1) determine the effect of varying test conditions (pH 6.0 to 8.0, divalent cation concentrations from 0.25 to 2X normal range, inoculum size from 10^3 to 10^7 CFU/ml, presence or absence of CO₂, and presence of blood in the test medium) on the *in vitro* activity of sulopenem; 2) compare agar dilution MICs to those obtained from broth microdilution, and 3) to determine short term stability of the drug at -20° C and -70° C.

Results: Wide variations in divalent cation concentration had little effect on the activity of sulopenem with the exception of a slight decrease in activity at high concentrations of calcium. Variations in pH, incubation atmosphere, and the presence of blood had little effect on sulopenem MICs. There was a slight inoculum effect in that the MICs of most study strains increased approximately 2- to 4-fold over a range of inoculum density of 10^3 through 10^7 CFU/ml. Excluding MRSA, sulopenem broth microdilution MICs compared favorably to agar dilution MICs when tested against staphylococci, streptococci and *Enterobacteriaceae*. The results were slightly more variable when tested against *H. influenzae*. As with other penems and carbapenems, sulopenem is relatively unstable when stored at -20° C but highly stable when the MIC trays are stored at -70° C or below for up to 6 months.

Conclusions: When performed within CLSI guidelines, minor variations in pH, divalent cation concentration, incubation in CO₂, and presence of blood in the test medium had little effect on the *in vitro* activity of sulopenem. Increasing inoculum size increased MICs by 2- to 4-fold. Agar dilution MICs compared favorably to broth microdilution. Sulopenem was stable up to 6 months when stored at -70° C or below.

F1-351

***In Vivo* Efficacy of Novel Prodrugs of Sulopenem**

A. MARRA, L. LAMB, I. MEDINA, D. GEORGE, J. O'DONNELL, A. MARFAT, T. GOOTZ;
Pfizer, Inc., Groton, CT.

Background: The antibacterial agent sulopenem is being developed to combat serious infections caused by multi-resistant bacterial pathogens. It has good *in vitro* activity against a broad range of Gram-negative and Gram-positive organisms and demonstrates potent *in vivo* efficacy when dosed via the subcutaneous route. Novel prodrugs of sulopenem, PF-00398899, PF-03709270, and PF-04064900, exhibit *in vivo* efficacy when administered orally in three different animal infection models. The organisms used to establish each infection were chosen for their resistance patterns to antibacterial drugs currently in use, including ESBL⁺ *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Methods: Murine septicemia models against *K. pneumoniae*, *E. coli* and *S. pneumoniae*, a murine respiratory tract infection model against *S. pneumoniae* and a gerbil otitis media model with *H. influenzae* were used to evaluate the efficacy of the prodrugs in comparison with marketed agents and subcutaneously-dosed sulopenem.

Results: In acute systemic infection models and in a respiratory tract infection model, sulopenem prodrugs were active in protecting mice from lethal infection. In the acute systemic infection model the prodrugs exhibited PD₅₀s of 9.0 - 88.3, 0.78 - 7.9, and 6.3 - 38.5 mg/kg against *K. pneumoniae*, *E. coli*, and *S. pneumoniae*, respectively. The PD₅₀s of the prodrugs in the respiratory model were 7.9 - 38.5 mg/kg. The prodrugs were also able to eradicate bacteria from a middle ear infection in Mongolian gerbils caused by *H. influenzae*, with ED₅₀s of 4.5 - 12.6 mg/kg.

Conclusions: All three oral prodrugs demonstrated efficacy comparable to that of sulopenem dosed subcutaneously against these infections.

F1-352

Biopharmaceutical-Based Pharmacokinetic Modeling of Sulopenem Prodrugs: Impact of Luminal Stability on Fraction of Dose Absorbed

J. O'DONNELL, K. BRIGHTY, J. ENGTRAKUL, M. FICHTNER, T. GOOTZ, J. HARDINK, A. HAZRA, L. LAMB, J. LO, A. MARFAT, A. MARRA, D. MCLEOD, R. MONAHAN, L. PRICE, W. SMITH, K. SOMA, S. SUTTON, J. WINTON; PGRD, Pfizer Inc, Groton, CT.

Background: PF-00398899, a lipophilic prodrug ester of sulopenem, was developed for oral administration. Although moderate bioavailability (F) was observed in preclinical species, only 11% F was achieved in humans. Upon further evaluation it was determined that intestinal stability of the prodrug was potentially poor, as suggested by *in vitro* incubations with porcine pancrelipase (PP) ($T_{1/2} < 2$ min). Using PP as an initial screen, prodrugs demonstrating $T_{1/2} > 15$ min were advanced to kinetic stability assessments in human intestinal juice (HIJ) in which Michaelis-Menten (M-M) parameters V_{max} and K_m were determined and utilized in a biopharmaceutical-based pharmacokinetic model (BBPK) to predict fraction of dose absorbed.

Methods: Prodrug degradation reactions (37 °C, 30 minutes) were initiated upon addition of substrate (10 μ M to 300 μ M) into buffered preparations of PP or HIJ. The amount of prodrug remaining as a percentage of compound at time = 0 was determined by LC/MS/MS. A first order degradation rate constant (k_{obs}) was determined by log-linear regression of the data. For HIJ incubations, M-M parameters were estimated from a log-linear plot of prodrug concentration vs. $k_{obs} \cdot V_{max}$ and K_m estimates were then utilized in a BBPK model to predict fraction of dose absorbed.

Results: BBPK model predictions for fraction of dose absorbed for prodrugs PF-00398899, PF-03709270, and PF-04064900 were 20%, 63%, and 82% for a 1200 mg dose. In clinical trials, oral F of 11%, 25%, and 39% were achieved with PF-00398899, PF-03709270, and PF-04064900, respectively.

Conclusion: 1) Assuming first pass extraction of 30 to 50%, model estimates of fraction of dose absorbed were in good agreement with observed clinical bioavailability for each prodrug. 2) Luminal stability of ester prodrugs is an important consideration in the development of these compounds.

F1-353

Pharmacokinetics (PK), Safety and Tolerability of Single Oral Doses of PF-03709270, with and without Co-administration of Probenecid

R. CHANDRA, A. HAZRA, T. SKOGERBOE, R. LABADIE, D. KIRBY, K. SOMA, M. DUNNE; Pfizer Global Res. & Dev., New London, CT.

Background: PF-03709270 is a novel oral prodrug of sulopenem (CP-70,429), a parenteral penem antibiotic being developed for hospital and community infections. Upon oral absorption, PF-03709270 is expected to be hydrolyzed, yielding active CP-70,429. The PK/pharmacodynamic determinant of efficacy for penems, i.e., Time above minimum inhibitory concentration (MIC), correlates with its efficacy. The target MIC values for CP-70,429 efficacy in community and hospital infections are 0.5 µg/mL and 1 µg/mL, respectively.

Methods: Nine healthy subjects received single doses of PF-03709270 (400 to 2000 mg), and a placebo in a 4-way crossover design. In a subsequent 3-way crossover study, 4 subjects received 600 mg of PF-03709270 alone and in combination with 500 and 1000 mg of probenecid. Safety was assessed by adverse event (AE), vital signs, heart rate, blood pressure, ECG, cardiac telemetry and safety lab monitoring. CP-70,429 plasma concentration-time data were analyzed by non-compartmental methods using WinNonlin v.3.2 (Pharsight®, Mountain View, CA).

Results: There were no discontinuations, serious or severe AEs, or significant ECG or lab abnormalities. All AEs were mild. CP-70,429 systemic exposure following PF-03709270 single doses increased in a dose dependent manner. The apparent terminal half-life ($t_{1/2}$) of CP-70,429 was dose independent. At a dose of 2000 mg, PF-03709270 produced CP-70,429 mean plasma exposures above the MIC targets of 0.5 µg/mL for 5.98 hrs, and above 1 µg/mL for 4.82 hrs. Co-administration of 500 and 1000 mg of probenecid with PF-03709270 increased the total mean AUC of CP-70,429 by 33.8 and 65.1%, respectively, when compared to PF-03709270 administered alone.

Conclusions: Oral PF-03709270 produced good exposures of CP-70,429 in terms of Time above MIC. Co-administration of probenecid increased the exposure and $t_{1/2}$ of CP-70,429. PF-03709270 was safe and well tolerated in both studies.

F1-354

Activity Profile of CXA-101 against Gram-Positive and Gram-Negative Pathogens by Broth and Agar Dilution

N. P. BROWN¹, C. M. PILLAR¹, D. F. SAHM¹, Y. GE²;

¹Eurofins Medinet, Inc., Anti-Infective Services, Herndon, VA, ²Calixa Therapeutics, Inc., San Diego, CA.

Background: CXA-101 (CXA) is a novel parenteral cephalosporin with excellent activity against *P. aeruginosa* (PA). In this study, the *in vitro* antibacterial profile of CXA was evaluated against Gram-negative (GN) and -positive (GP) organisms.

Methods: GP (n = 310; 70 *S. aureus*, 100 *S. pneumoniae*, 100 β -hemolytic strep, 40 enterococci) and GN (n = 785; 300 PA, 20 *B. cepacia*, 365 Enterobacteriaceae [EN], 50 *M. catarrhalis*, 50 *H. influenzae*) isolates collected from 2006-2007 were centrally tested against CXA and comparators by broth microdilution while a selection of isolates were concurrently tested by agar dilution (CLSI M7-A7).

Results: CXA showed little activity against *S. aureus* and enterococci (MIC₉₀ \geq 64 μ g/ml), but had activity by MIC₅₀/MIC₉₀ (μ g/ml) similar to ceftazidime (CTZ) against *S. pneumoniae* (\leq 0.12/4) and β -streptococci (Group A: \leq 0.12/0.5; Group B: 0.5/1). CXA had potent activity by MIC₅₀/MIC₉₀ (μ g/ml) against EN overall (0.25/1), similar to CTZ (0.12/2) and lower than cefepime (CEF [\leq 0.06/0.25]). Similar to other cephalosporins, CXA was highly active by MIC₅₀/MIC₉₀ (μ g/ml) against *H. influenzae* (\leq 0.12/0.25) and *M. catarrhalis* (\leq 0.12/0.5). Against PA, activity of CXA by MIC₅₀/MIC₉₀ (1/2) was several-fold higher than CTZ (2/32), CEF (4/16), and imipenem (4/16). Both CXA and CTZ had an MIC₅₀/MIC₉₀ of 4/32 μ g/ml against *B. cepacia*, lower than CEF (32/>32). 99% of PA tested had CXA MICs \leq 8 μ g/ml, the best among all anti-*pseudomonal* drugs tested. Among the GN and GP, broth microdilution results correlated well with agar dilution results with MICs within 1 doubling-dilution for 90% of the isolates.

Conclusions: CXA was the most potent *in vitro* β -lactam against PA highlighting its potential as an anti-*pseudomonal* β -lactam. It also displayed activity similar to CTZ and CEF against other GNs and streptococci. Good correlation between broth and agar MICs illustrate that agar dilution is suitable for susceptibility testing of CXA.

F1-355

Activity of Cephalosporin CXA-101 (FR264205) vs. *P. aeruginosa*

S. MUSHTAQ ¹, M. WARNER¹, Y. GE ², D. M. LIVERMORE ¹;

¹HPA Ctr. for Infections, London, United Kingdom, ²Calixa Inc, San Diego, CA.

Background: *P. aeruginosa* can become resistant to ceftazidime -generally the most active cephalosporin vs. the species- via mutational derepression of AmpC or up-regulation of efflux. More rarely, resistance arises by acquisition of extended-spectrum or metallo- β -lactamases. Mutational resistance is most prevalent among cystic fibrosis (CF) isolates, reflecting antibiotic pressure. We examined the activity of CXA-101 vs. *P. aeruginosa* strains with these mechanisms.

Methods: *P. aeruginosa* tested includes clinical isolates or laboratory strains, many with characterized resistance mechanisms. MICs were determined by the CLSI agar dilution method.

Results: Against 'typical' *P. aeruginosa* strains without acquired resistance, MICs of CXA-101 were 0.25-0.5 mg/L, compared with 1-2 mg/L ceftazidime. Against strains with up-regulated efflux or total derepression of AmpC, MICs of CXA-101 were 0.5-1 mg/L and 4 mg/L, respectively, compared with 2-16 mg/L and 32-128 mg/L, respectively, for ceftazidime; thus, for both prevalent resistance types, CXA-101 retained good activity. Resistance to CXA-101, with MICs of ≥ 32 mg/L, arose for organisms with OXA-ESBLs (e.g. OXA-11 and 14), PER-1, VEB-1, VIM and IMP β -lactamase, all of which also conferred ceftazidime resistance. Low-level resistance (MIC 8 mg/L) to CXA-101 arose in transconjugants with OXA-2 or -3 enzymes, although only the former enzyme significantly raised the ceftazidime MIC. The MICs of CXA-101 were 2- to 16- fold below those of ceftazidime for multi-resistant *P. aeruginosa* from CF patients, but still ranged up to 128 mg/L; these isolates are believed to have multiple mechanisms.

Conclusions: CXA-101 is a more active antipseudomonal cephalosporin than ceftazidime, with good activity vs. isolates with derepression of AmpC or up-regulated efflux; activity was compromised by several rarer β -lactamases; also by the complex combinations of mechanism found in some CF isolates.

F1-356

Activity of cephalosporin CXA-101 (FR264205) with β -Lactamase Inhibitors vs. Enterobacteriaceae

S. MUSHTAQ ¹, M. WARNER¹, Y. GE ², D. M. LIVERMORE ¹;

¹HPA Ctr. for Infections, London, United Kingdom, ²Calixa Inc, San Diego, CA.

Background: CXA-101 is a novel parenteral oxyimino-cephalosporin 2-8-fold more active than ceftazidime vs. *P. aeruginosa*. We examined its behaviour vs. isolates and predictor panels of Enterobacteriaceae with characterized β -lactamases.

Methods: MIC were determined by the CLSI agar dilution method with ceftazidime as a comparator. Both cephalosporins were tested alone and in combination with clavulanate and tazobactam at 4 mg/L.

Results: MICs of CXA-101 and ceftazidime for Enterobacteriaceae strains without acquired resistances, or with acquired penicillinases, were clustered around 0.12 - 0.5 mg/L, irrespective of species. Derepression of AmpC raised the MICs of CXA-101 to 8-32 mg/L for *Enterobacter* spp and *C. freundii* and to 2-16 mg/L for *Serratia* spp. or *M. morgani*; corresponding MICs of ceftazidime for these groups were 16-64 mg/L and 2-16 mg/L, respectively. AmpC-mediated resistance to CXA-101 or ceftazidime was variably reversed by tazobactam, never by clavulanate. ESBLs markedly raised the MICs of CXA-101 to >4 mg/L, with the compound affected more than ceftazidime by several common types, including CTX-M-3 and -14. Both compounds were similarly affected by CTX-M-15, now the predominant ESBL in much of the world outside the USA. ESBL-mediated resistance to CXA-101 or ceftazidime was reversed by clavulanate, with combination MICs \leq 2 mg/L; reversal of resistance by tazobactam also was seen for most ESBL producers, though with a few exceptions, not clearly related to enzyme type. Clavulanate- and tazobactam- independent resistance to CXA-101 was conferred by IMP and KPC enzymes, but by not SME or IMI types.

Conclusions: CXA-101 - a highly potent antipseudomonal cephalosporin - behaved as a typical extended-spectrum oxyimino cephalosporin vs. Enterobacteriaceae. ESBL-mediated resistance was overcome by combination with clavulanate or, less reliably, tazobactam.

F1-357

Effect of Various Testing Parameters on the Activity of CXA-101 by Broth Microdilution

N. P. BROWN¹, C. M. PILLAR¹, D. F. SAHM¹, Y. GE²;

¹Eurofins Medinet, Inc., Anti-Infective Services, Herndon, VA, ²Calixa Therapeutics, Inc., San Diego, CA.

Background: CXA-101 (CXA) is an investigational cephalosporin with potent *in vitro* activity against *P. aeruginosa* (PA). It is important to understand the impact of a variety of *in vitro* testing conditions on the *in vitro* activity of antibiotics. This study addresses the effect of serum, pH, inoculum size, divalent cation concentration, and testing media on the activity of CXA against select pathogens.

Methods: Two isolates each of *S. aureus* (SA), *S. pneumoniae* (SP), *E. coli* (EC), *K. pneumoniae* (KP), and PA were tested for MIC by broth microdilution against CXA and ceftazidime (CTZ) under standard conditions (CLSI M100-S17, M7-A7). Concurrently, the effect of varied pH (pH 5, 6, 8), serum (10 and 50%), inocula (5×10^4 and 5×10^6 CFU/ml), divalent cations (Ca^{2+} at 50 $\mu\text{g/ml}$), and media (cation-adjusted Mueller-Hinton Broth [CAMHB], CAMHB + lysed horse blood [LHB], and *Haemophilus* testing medium [HTM]) on the activity of CXA was evaluated.

Results: Human serum (20 or 50%) had little to no effect on the activity of CXA overall though a drop in CXA MICs was noted against 1 PA isolate (4-fold) and 1 EC isolate (2 to 8-fold). Altering the inoculum concentrations to 5×10^4 CFU/ml and 5×10^6 CFU/ml did not affect the overall activity of CXA. CAMHB + LHB or HTM did not affect the activity of CXA against SA, PA, EC, and KP. Testing SP with HTM did not affect CXA MICs, while testing SP in CAMHB alone either did not support growth of the isolate or resulted in lower MICs by 2-fold for CXA, which is likely due to poor growth of the organism in the absence of LHB. Testing at 50 mg/L Ca^{2+} did not affect the activity of CXA against any of the isolates tested. pH did not significantly affect the activity of CXA overall, though higher MICs for CXA were noted at low pH (pH 5) against EC and KP.

Conclusions: Variations in pH, inoculum size, testing medium, divalent cation concentration, or serum concentration had little to no effect on the antimicrobial activity of CXA against the gram-positive and -negative pathogens tested.

F1-358

Mode of Action of CXA-101 Based on Minimum Bactericidal Concentration (MBC) Analysis and Timekill Kinetic (TK) Analysis

N. P. BROWN¹, C. M. PILLAR¹, D. F. SAHM¹, Y. GE²;

¹Eurofins Medinet, Inc., Anti-Infective Services, Herndon, VA, ²Calixa Therapeutics, Inc., San Diego, CA.

Background: CXA-101 (CXA) is a novel parenteral cephalosporin which has shown to have excellent activity against *P. aeruginosa* (PA). In order to better understand the cidal mode of action of CXA, this study evaluated the MBC and TK of CXA against isolates of *S. pneumoniae* (SP), PA (including resistant [R] isolates), *B. cepacia* (BC), *E. coli* (EC), *K. pneumoniae* (KP), and *M. catarrhalis* (MC).

Methods: The MIC and MBC of CXA, ceftazidime (CTZ), cefepime (CEF), and imipenem (IMP) was determined in accordance with CLSI M7-A7 and M26-A by broth microdilution with the MBC defined as the concentration where a 3-log kill was observed. 10 SP, 20 PA, 10 BC, 10 EC, 10 KP, and 5 MC were evaluated. TK of CXA were also evaluated (CLSI M26-A) against recent clinical isolates (1 SP; 4 PA [including CTZ-R isolates]; 2 BC; 2 EC; 2 KP; 1 MC) at 1X, 4X, and 8X the MIC. Viability was assessed and cidal activity was defined as ³ 3-log₁₀ reduction in CFU at 24 hr.

Results: CXA MBC:MIC ratios ranged from 1-2 against most isolates of the evaluated species, excluding PA where MBC:MIC ratios of 2-4 was observed only for 45% of PA (a result also observed with CEF and CTZ). Based on TK data, CXA was cidal against all evaluated isolates at 4X and 8X the MIC with the exception of KP where CXA was cidal at 8X the MIC. The cidal activity of CXA against PA was not altered against CTZ-R isolates. A 3-log kill was generally apparent between 6-8 hr and as early as 4 hr in some instances. At 1X the MIC, an initial kill similar to that observed at 4X and 8X the MIC was observed at early time points, however by 24 hr all isolates had regrown, excluding MC where CXA was cidal at 1X the MIC.

Conclusions: The cidal activity of CXA by MBC analysis, with MBC:MIC ratios of ≤ 2 against a majority of the evaluated isolates, was validated by TK data, especially upon exposure to multiples of the MIC as shown in the TK study. These findings indicate that CXA, similar to other β -lactams, is a potent cidal agent against most target pathogens.

F1-359

Effect of Inoculum Size, Medium, pH, and Human Serum on Activity of SMP-601 Against Key Pathogens

K. KANAZAWA, K. TAKEMOTO, K. FUJIMOTO, K. EGUCHI, T. UDA; Dainippon Sumitomo Pharma, Osaka, Japan.

Background: SM is a novel parenteral carbapenem (CB) with a unique spectrum including anti-MRSA and VRE activity. In this study, we investigated the effects of inoculum size, medium, pH, and human serum on the activity of SM against key pathogens, mainly staphylococci and enterococci.

Method: Eleven standard strains (*S. aureus* (MSSA, MRSA), *S. epidermidis* (MSSE), *E. faecalis* (Efs), *E. fecium* (Efm), *E. coli* (Ec), *K. pneumoniae* (Kp), *P. aeruginosa* (Pa)) were subjected to MIC testing with SM and other relevant β -lactams (meropenem (ME), imipenem (IP), cefepime (CE), piperacillin/tazobactam (PT)). MIC tests were performed by the agar dilution method according to CLSI. Test conditions were as follows: inoculum size, 10^5 , 10^6 , 10^7 , 10^8 CFU/mL; medium, MHA, NA, HIA, BHIA (Difco), SDA-N (Nissui), TSA (BBL); pH, 5.5, 6.5, 7.5, 8.5; human serum %, 0, 10, 20, 50%.

Results: The MICs of each agent at various inoculum sizes are shown in the table.

The MIC of SM against each strain was not significantly influenced by inoculum size except that against Pa. The MICs of all agents were affected by medium composition; however, the effects on the MICs of SM showed a striking similarity to those of other CBs, ME and IP. Most of the MICs of SM were 2- to 8-fold lower under acidic conditions (pH 5.5 and 6.5), and was 2- to 8-fold higher under alkaline condition (pH 8.5) than at pH 7.5. The same pH effects were also observed in other CBs. Addition of human serum slightly reduced the activities of 3 CBs, including SM. In contrast to CBs, MICs of CE and PT with human serum were 2- to 8-fold lower than those in MHA.

Conclusion: The effects of these factors on the activity of SM against these pathogens were intrinsically equivalent to those of existing CBs.

Test strain	MIC at each inoculum size of $10^5/10^8$ CFU/mL [mg/L]			
	SM	ME	CE	PT
MSSA ATCC6538P	0.008/0.015	0.03/0.06	0.5/2	0.5/0.5
MSSA ATCC29213	0.015/0.015	0.06/0.12	1/2	1/1
MRSA ATCC43300	0.12/0.25	0.5/2	64/128	4/32
MSSE ATCC14990	£0.004/0.008	0.03/0.06	0.25/1	0.12/0.12
Efs ATCC19433	1/1	4/4	16/32	4/8
Efs ATCC29212	0.5/0.5	4/4	16/64	4/8
Efm ATCC19434	0.25/0.5	8/16	>128/>128	16/16
Ec ATCC25922	0.25/0.25	0.015/0.03	0.03/0.06	2/8
Ec ATCC11775	0.12/0.25	0.015/0.015	0.03/0.03	2/2
Kp ATCC10031	0.06/0.12	0.03/0.06	0.015/0.03	0.06/0.12
Pa NBRC3451	4/16	0.25/0.5	0.5/1	4/8

F1-360

In Vitro and In Vivo Efficacy of SMP-601 (SMP; PZ-601), a Novel Anti-MRSA Carbapenem, against Clinical Isolates of MRSA in Japan

K. FUJIMOTO, T. UDA, K. KANAZAWA; Dainippon Sumitomo Pharma, Osaka, Japan.

Background: SMP is a new carbapenem with potent activity against various resistant pathogens, including MRSA, PRSP and VRE. At the 47th ICAAC, we presented *in vitro* antibacterial activity against clinical isolates of gram-positive and -negative bacteria. SMP showed remarkably excellent *in vitro* anti-MRSA activity than other β -lactam agents. In this study, we investigated the *in vitro* bactericidal activity and *in vivo* therapeutic efficacy of SMP against clinical isolates of MRSA as compared to other anti-MRSA agents.

Methods: Twenty-one clinical isolates of MRSA were collected in Japan. MICs, MBCs and Time-kill (TK) assay were conducted according to CLSI guidelines. TK assay was evaluated at 1/4, 1, 4, and 16 MIC of SMP and other anti-MRSA agents at time intervals of 0, 1, 2, 4, 6, and 8h. The *in vivo* efficacy was investigated in a systemic infection model using immunocompromised mice. Drugs were subcutaneously administered 2h after infection.

Results: The MIC/MBC ratio of each agent against 21 isolates of MRSA was as follows: SMP, 1-4; vancomycin (VC), 1->256; teicoplanin (TE), 1->256; linezolid (LZ), >32; and arbekacin (AB), 1-16. In the TK assay, SMP showed excellent bactericidal activity against MRSA SP-12249 at 1 to 16 MIC. SMP showed stronger bactericidal activity than VC, TE, and LZ, and comparable activity to that of AB. The *in vivo* ED₅₀ of SMP, VC, and LZ against infection with MRSA SP-12249 was 4.73, 6.00, and 7.44 mg/kg, respectively.

Conclusions: Against clinical isolated MRSA in Japan, SMP showed strikingly excellent *in vitro* bactericidal activity in comparison with other anti-MRSA agents. SMP also showed an excellent *in vivo* therapeutic effect, reflecting its potent *in vitro* antibactericidal activity.

In vitro activity and *in vivo* efficacy of each drug against MRSA SP-12249

Drugs	MIC / MBC (mg/mL)	Reduction of log CFU/mL at 4 MIC in TK assay				<i>In vivo</i> ED ₅₀ (mg/kg)
		2h	4h	6h	8h	
SMP	2 / 2	2.8	3.4	3.5	5.2	4.73
VC	2 / 4	0.7	1.7	1.9	1.9	6.00
LZ	2 / >128	0.5	0.8	1.2	1.7	7.44
AB	1 / 8	2.7	3.7	5.2	5.2	-

F1-361

Post-antibiotic Effect (PAE) of SMP-601 (PZ-601) Against *Staphylococcus aureus* including MRSA and VISA

T. UDA, K. TAKEMOTO, K. FUJIMOTO, K. EGUCHI, K. KANAZAWA; Dainippon Sumitomo Pharma, Osaka, Japan.

Background: SMP-601 is a novel parenteral carbapenem with an unique spectrum including anti-MRSA, VRE, and PRSP activity. In this study, we investigated the in vitro PAE of SMP-601 against *S. aureus*, mainly MRSA.

Methods: Three standard strains of *S. aureus* (MSSA, ATCC6538P; MRSA, ATCC33592; MRSA/hetero-VISA, ATCC700698 (Mu3)) and a clinical isolate of MRSA (SP-12249) were subjected to PAE testing with SMP-601 and other relevant anti-MRSA agents (vancomycin (VCM), teicoplanin (TEIC), linezolid (LZD), arbekacin (ABK)). MIC tests were performed by the microbroth dilution method according to CLSI guidelines. In vitro PAE test conditions were as follows: inoculum size, 10^6 CFU/mL; medium, CAMHB (Difco); antibiotic exposure, at 4x MIC at 35°C for 2h; antibiotic removal, broth dilution and centrifugation washing.

Results: The MICs and PAEs of each agent are shown in the table. The MICs of SMP-601 varied between 0.008 and 4 mg/L. The PAEs of SM were all between 0.58 and 2.64 h. TEIC showed the longest PAE (>2.73 h) among the 5 agents tested. The PAEs of VCM (1.58 - >3.42 h) and LZD (1.39 - 2.77 h) were equivalent to those of SMP-601. In contrast to these 4 agents, PAEs of ABK were remarkably short (-0.23 - 0.98 h).

Conclusions: The PAEs of SMP-601 against *S. aureus* were equivalent to those of VCM and LZD, longer than those of ABK, and shorter than those of TEIC. The excellent pharmacokinetic profile in humans and strong bactericidal activity against MRSA together with the PAE found in this study support the promising therapeutic potency of SMP-601 against various MRSA infections.

Test strain	In vitro PAE [h] (by exposure at 4x MIC for 2h, n=2) (MIC: mg/L)				
	SMP-601	VCM	TEIC	LZD	ABK
MSSA ATCC6538P (FDA209P)	1.14, 1.68 (0.008)	1.59, 1.58 (0.5)	>4.33, >4.17 (0.25)	2.46, 2.76 (2)	0.39, 0.20 (0.25)
MRSA ATCC33592	0.58, 0.81 (0.5)	>3.42, >3.04 (1)	>3.42, >3.04 (1)	1.47, 1.39 (2)	0.46, 0.66 (0.5)
MRSA/hetero-VISA ATCC700698 (Mu3)	1.88, 2.62 (4)	2.69, 2.00 (1)	>3.05, >2.73 (16)	2.77, 2.12 (2)	-0.09, -0.23 (2)
MRSA SP-12249	2.35, 2.64 (1)	2.69, 3.10 (1)	>3.87, >3.76 (1)	2.70, 2.08 (2)	0.94, 0.98 (1)

F1-1164

Activity of Novel Monobactam BAL30072 against Multiresistant Non-Fermenters

S. MUSHTAQ, M. WARNER, D. LIVERMORE;
HPA Ctr. for Infections, London, United Kingdom.

Background: BAL 30072 is a novel siderophore monobactam. We tested its activity vs panels of pseudomonads and *Acinetobacter* spp.

Methods: MICs were determined on Mueller-Hinton agar supplemented with 2,2' bipyridyl to induce TonB-mediated transport; comparators included aztreonam and BAL19764 (Syn/PTX2416) as a reference catecholic monobactam known to exploit TonB-mediated transport. Test panels, comprising clinical isolates and genetic constructs were biased towards isolates with multiresistance to other agents.

Results: BAL30072 was active vs. 73, 83, 86 and 89% of 200 carbapenemase producing *A. baumannii* at 1, 2, 4 and 8 mg/L respectively, with activity relating to the producer clone rather than the carbapenemase (OXA-23, -40, 51 or 58-like, or IMP-1) produced. MICs of BAL30072 ranged from 0.015 to 64 mg/L against 87 *P. aeruginosa* isolates that lacked derepression of AmpC or acquired β -lactamases but which varied in their level of intrinsic (i.e. efflux-type) resistance, with 66% of MIC values between 1-8 mg/L; MICs for this collection were more closely related to those of BAL19764 than aztreonam, implying utilisation of TonB-mediated transport and at least partial evasion or overloading of efflux. MICs for mutants were raised from 2-4 mg/L to 16 mg/L by total derepression of AmpC and those for transconjugants to 8-16 mg/L by various uncommon OXA β -lactamases, and to 32 mg/L by PER-1. Only 37% of 47 multiresistant *P. aeruginosa* isolates from cystic fibrosis were susceptible to BAL30072 at 8 mg/L; however 76% of 21 *B. cepacia* complex isolates were susceptible, with 71% susceptible at ≤ 1 mg/L; by contrast 81% of these *P. aeruginosa* and 90% of the *B. cepacia* complex isolates were resistant to aztreonam 8 mg/L.

Conclusion: BAL 30072 had impressive activity vs carbapenemase-producing *A. baumannii* and against *B. cepacia* complex isolates from cystic fibrosis; activity against multiresistant *P. aeruginosa* was more variable, but remained good vs many isolates with broad-spectrum efflux-type resistance.

F1-1165

Bal 30072 a Siderophore Monobactam with Extended Activity Against Multidrug Resistant (MDR) *Acinetobacter baumannii*

A. M. HUJER¹, K. M. HUJER¹, M. G. P. PAGE², R. A. BONOMO¹;

¹VAMC, Cleveland, OH, ²Basilea Pharmaceutica Intl. Ltd., Basel, Switzerland.

Background: Among MDR-GNB, *Acinetobacter baumannii* that are resistant to extended-spectrum cephalosporins (e.g., ceftazidime and cefepime) and carbapenems (e.g., imipenem, meropenem, and ertapenem) are becoming a major global problem. The future development of novel beta-lactams must target the “complex beta-lactamase background” of these contemporary Gram-negative bacteria. BAL30072 is a monobactam that possesses a siderophore side chain. To test the efficacy of BAL30072, we performed MICs against MDR *Acinetobacter baumannii*. These strains were chosen because the genetic determinants of beta-lactam resistance were previously defined.

Methods: Minimum inhibitory concentrations, MICs, were determined according to Clinical Laboratory Standards Institute (CLSI) methods. We used an MIC \leq 8 mg/L as a breakpoint for BAL30072. For all other antibiotics the susceptibility is based on the CLSI breakpoint.

Results: Compared to meropenem, imipenem, cefepime and tigecycline, BAL30072 was the most potent antibiotic tested (median MIC 0.5 mg/L) and had activity superior to comparator antibiotics against this panel of strains (Table). A small number of strains (10/124) exhibited very high MICs for BAL30072 .

Conclusions: BAL30072 demonstrated significantly better activity than imipenem vs. highly resistant isolates of *A. baumannii*, including strains possessing *bla*ADC and *bla*OXA-23-like genes.

Table: Activity of BAL30072 and comparators against multi-resistant <i>Acinetobacter</i>	Drug	Minimum inhibitory concentration (mg/L)			Susceptible* (%)
		MIC ₅₀	MIC ₉₀	Range	
	BAL30072	0.5	8	\leq 0.06 - $>$ 64	(91)
	Meropenem	1	32	\leq 0.06 - $>$ 64	62
	Imipenem	1	32	\leq 0.06 - $>$ 64	66
	Cefepime	16	32	\leq 0.06 - $>$ 64	31
	Tigecycline	2	4	0.25 - 16	82

* The susceptibility to BAL30072 is given in parentheses for the percentage of strains with an MIC \leq 8 mg/L.

F1-1166

Efficacy of BAL30376, a New Monobactam/ beta-Lactamase Inhibitor Combination, against *Pseudomonas aeruginosa* (PA)

O. HERMESH¹, M. G. P. PAGE², Y. CARMELI¹, S. NAVON-VENEZIA¹;

¹Tel Aviv Sourasky Med. Ctr., Tel-Aviv, Israel, ²Basilea Pharmaceutica Intl. Ltd, Basel, Switzerland.

Background: PA is a difficult to treat common nosocomial pathogen often affecting immunocompromised patients. BAL30376 is a new antimicrobial combination composed of a monobactam, a class C beta lactamase inhibitor and clavulanic acid. We evaluated the *in vitro* and the *in vivo* activity of this compound against PA.

Methods: The *in vitro* activity of BAL30376 was tested against 102 resistant PA strains [60% resistant to ceftazidime (CAZ)]. MICs were compared to aztreonam (AZT), beta lactams (blac) and blac/inhibitors combinations by agar dilution. Time kill studies were performed. The *in vivo* efficacy and dose response were performed using a neutropenic mice peritonitis sepsis model. PA infecting inocula ranged from 5×10^2 - 1×10^4 cfu/mouse (0.6-12XLD50) and BAL30376 was administered 3 times a day for 3 days (12.5 to 100 mg/Kg). Survival was monitored and compared with a placebo and meropenem (MER) treated groups.

Results: MIC₅₀ and MIC₉₀ of BAL30376 against PA were 2 and 8 mg/L, respectively, 8 fold lower than MIC50 of AZT, CAZ, and cefepime, 2-8 folds lower than MER and 8-32 fold lower than piperacillin/tazobactam. Bactericidal activity revealed that BAL30376 at 4 and 16XMIC exhibited antibacterial activity (1.8-2 log₁₀ cfu/mL decrease) gradually over the first 8h. In vivo experiments in mice infected intraperitoneally with various PA inocula disclosed that at doses of 50 and 100 mg/kg BAL30376 conferred full protection (90-100% survival), similarly to MER, and opposed to placebo group (0-33% survival, $p < 0.001$). At 25 mg/Kg BAL30376 was effective against doses up to 6XLD50 ($p = 0.05$). Lower doses of BAL30376 (<25 mg/Kg) were moderately protective.

Conclusions: BAL30376 is an active antibacterial agent against resistant strains of PA, with promising spectrum of activity and bactericidal effect. It is highly effective treatment against infection caused by PA, as demonstrated in murine lethal peritonitis and sepsis model.

F1-1167

In Vitro Activity of BAL30376 and its Components vs. Multiresistant Enterobacteriaceae

S. MUSHTAQ, **M. WARNER**, D. M. LIVERMORE;
HPA Ctr. for Infections, London, United Kingdom.

Background: BAL 30376 combines a catechol monobactam BAL19764 (Syn/PTX 2416) with both a bridged monobactam (BAL 29880) to inhibit AmpC enzymes and clavulanate to inhibit ESBLs. We tested component activity vs. predictor panels of Enterobacteriaceae isolates, transconjugants and mutants.

Methods: MICs were determined on Mueller-Hinton agar supplemented with 2,2' bipyridyl to chelate Fe⁺⁺⁺ and induce TonB-mediated uptake; BAL 29880 was added at 4 mg/L and clavulanate at 2 mg/L; alternatively a 5:3:1 BAL19764: BAL29880: clavulanate ratio was used.

Results: Alone, monobactam BAL19764 had MICs \leq 1 mg/L for 94 % of 101 Enterobacteriaceae without cephalosporin resistance, though a few isolates exhibited MICs up to 8 mg/L. MICs of BAL19764 were >1 mg/L for $>90\%$ of isolates with ESBLs (n=260) or high-level AmpC (n=155). Addition of clavulanate reduced MICs of BAL19764 to \leq 1 mg/L for $>74\%$ of ESBL producers; addition of BAL29880 to BAL19764 reduced MICs for $>65\%$ of AmpC producers. The BAL 30376 triple combination had MICs <1 mg/L for 75% of isolates with either enzyme. A few ESBL and AmpC isolates -some otherwise unexceptional members of widespread ESBL clones- required much higher MICs, suggesting some unusual co-determinant of susceptibility. MICs of BAL19764 for metallo- β -lactamase producers (n=5) ranged from 0.25 to >128 mg/L; those of BAL30376 from 0.12 to 16 mg/L MICs of BAL30376 for isolates with KPC enzymes were >128 mg/L; those for isolates with carbapenem resistance contingent on combination of AmpC or ESBL and porin loss were >1 mg/L in 59%, and >128 mg/L, in 22% of cases.

Conclusions: BAL 30376 overcame most AmpC- or ESBL- mediated resistance, though less consistently than a carbapenem. Unlike a carbapenem, BAL30376 also was active vs. most metallo- β -lactamase producers. High MICs for a few isolates, independent of β -lactamase type, may reflect lesions in TonB-mediated transport.

F1-1168

***In Vitro* Activities of Piperacillin in Combination with BLI-489 and Comparative Antibacterial Agents Against Recent Clinical Isolates**

P. J. PETERSEN, C. H. JONES, A. M. VENKATESAN, P. A. BRADFORD;
Wyeth Res., Pearl River, NY.

Background: The novel bicyclic penem inhibitor, BLI-489 (BLI) has demonstrated activity as an inhibitor against Class A (including ESBLs), and Class D as well as Class C β -lactamase enzymes. This new β -lactamase inhibitor offers a broader spectrum of activity compared to the current commercial inhibitors. This study was performed to evaluate BLI in combination with piperacillin (PIP) against a large and diverse collection of recent clinical isolates (N=1744).

Methods: The MICs of the antibiotics were determined by broth microdilution as recommended by the CLSI. The activity of the combination of PIP:BLI was determined with a constant 4 $\mu\text{g/ml}$ of BLI. The PIP:tazobactam (TZB) interpretation criteria was used for both PIP:BLI and PIP:TZB.

Results: The combination of PIP: BLI equaled or exceeded the activity demonstrated by PIP:TZB against all isolates tested. Approximately 54% of the enteric bacilli tested were non-susceptible to PIP alone. However, 91% of these PIP non-susceptible strains were susceptible to PIP:BLI (MIC₉₀s 0.5 - >128 $\mu\text{g/ml}$), in contrast, only 66% were susceptible to PIP:TZB (MIC₉₀s 2 - >128 $\mu\text{g/ml}$). The PIP:BLI combination also demonstrated improved activity (80% susceptible) over PIP:TZB against the problematic ESBL and AmpC producing strains. The more fastidious gram-negative organisms, *H. influenzae*, *H. parainfluenzae* and *M. catarrhalis*, were equally susceptible to the PIP:BLI combination (MIC₉₀s ≤ 0.004 - 0.015 $\mu\text{g/ml}$). Potent activity was demonstrated by PIP:BLI against the non-fermentative bacteria *B. cepacia*, *Acinetobacter* spp. and *P. aeruginosa* (MIC₉₀s 2, 16 and 64 $\mu\text{g/ml}$, respectively). The combination of PIP: BLI effectively enhanced the activity of PIP against the majority of gram-positive isolates tested.

Conclusions: The in vitro activity of PIP:BLI and the clear advantage demonstrated over PIP:TZB against a wide variety of bacteria, including ESBL and AmpC producing strains, makes BLI-489 a strong candidate for further development.

F1-1169

In Vitro Activities of Piperacillin in Combination with the Penem β -lactamase Inhibitor BLI-489 by Time-Kill Kinetics Studies

P. J. PETERSEN, C. H. JONES, A. M. VENKATESAN, P. A. BRADFORD;
Wyeth Res., Pearl River, NY.

Background: The β -lactamase inhibitor, BLI-489 (BLI), has shown *in vitro* activity against molecular Class A (including ESBLs), D and C β -lactamase enzymes. In this study the *in vitro* activities of the combination of piperacillin (PIP) and BLI or tazobactam (TZB) were determined by time kill kinetics against ten well-characterized β -lactamase producing organisms.

Methods: Time-kill assays were performed by the broth macrodilution method, as suggested by the CLSI guidelines. Flasks containing 50 mL of MHB with the appropriate antimicrobial agent were inoculated with 50 mL of test organism to a density of approximately 10^6 CFU/mL. Aliquots were removed, diluted and spiral plated to determine viable counts.

Results: The combination of PIP:BLI, at 4 x MIC, demonstrated a 6 hour reduction in inoculum of 1.7 to 2.5 \log_{10} CFU/ml. PIP:BLI with an average decrease of 2.4 \log_{10} CFU/ml, significantly reduced the initial inoculum of a Class A producing *E. coli* (TEM-1) and *K. pneumoniae* (SHV-11). PIP:BLI also demonstrated a average reduction of 2.2 \log_{10} CFU/ml against strains producing ESBLs (TEM-10 or SHV-5 or CTX-M-5). In contrast, PIP:TZB at the same concentration failed to reduce the original inoculum for the SHV-5 and CTX-M-5 producing pathogens. In addition, an average reduction of 2.3 \log_{10} CFU/ml of viable bacterial counts was shown by PIP:BLI against strains producing Class C (AmpC or ACT-1) or Class D (OXA-1) enzymes. The PIP:TZB combination was not effective in maintaining the original inoculum concentration of the Class C producing isolates. Although regrowth was observed for some of the isolates tested by the 24 hour time point, the clinical relevance of this phenomenon is unknown.

Conclusions: The PIP:BLI combinations demonstrated an average 2.2 \log_{10} CFU/ml decrease in the initial inoculum by the 6 hour time point against the ten β -lactamase producing strains tested. The PIP:BLI-489 combination offers an advantage over the current commercial inhibitors and warrants further development.

F1-1170

ME-1071, a Novel Metallo- β -Lactamase Inhibitor: Inhibition Mechanism and In Vitro Synergistic Activity Against MBL-producing Gram-Negative Pathogens

J. D. DOCQUIER, F. DE LUCA, L. BORGIANNI, G. M. ROSSOLINI;
Univ. of Siena, Siena, Italy.

Background: The worldwide emergence of metallo-beta-lactamase- (MBL-) producing Gram-negative pathogens represents a worrisome clinical issue, as they commonly exhibit a multidrug-resistance phenotype and are not susceptible to conventional beta-lactamase inhibitor-beta-lactam combinations, emphasizing the clinical need for clinically useful MBL inhibitors. Here, we describe the inhibition properties and synergistic activity of ME-1071, a new MBL inhibitor developed by Meiji Seika Kaisha co., Japan.

Methods: The inhibition mechanism of ME-1071 was investigated with purified VIM-1, VIM-2 and VIM-4 MBLs using the Dixon plot and the inhibition constants (K_i s) computed accordingly. MIC values of imipenem (IPM), meropenem (MEM), biapenem (BIA), doripenem (DPM), ceftazidime (CAZ) and cefepime (FEP) were determined for MBL-producing clinical isolates (including *P. aeruginosa*, *A. baumannii*, *Achromobacter xylosoxidans* and *Enterobacteriaceae*) and recombinant *E. coli* strains carrying various cloned MBL genes using both agar dilution and broth microdilution methods, as recommended by CLSI, in the absence and presence of 32 μ g/ml ME-1071.

Results: In enzyme assays, ME-1071 appeared to be a competitive inhibitor of the three tested VIM-variants (K_i values; 5.5, 0.81 and 0.18 μ M for VIM-1, VIM-2 and VIM-4, respectively). With *E. coli* transformants producing different IMP, VIM or SIM-1 MBLs, BIA was the most effective antibiotic while the addition of ME-1071 restored full susceptibility to all tested agents, with observed MIC decrease up to >100-fold. ME-1071 also showed antibiotic potentiation on clinical isolates, and the overall best synergistic effect was observed with MEM, BIA and DPM.

Conclusions: ME-1071 is a potent inhibitor of clinically-relevant MBLs able to revert beta-lactam resistance *in vitro*. ME-1071 represents a valuable compound for subsequent optimization and development of clinically-useful MBL inhibitors.

F1-1171

Activity Of Me1071, a Metallo- β -lactamase (MBL) Inhibitor, in Tests with American Plasmid-mediated MBLs

E. SMITH MOLAND, K. S. THOMSON;

Creighton Univ., Omaha, NE.

Background: This study investigated the activity of ME1071, a competitive inhibitor of plasmid-encoded MBLs, in combination with imipenem (IPM), meropenem (MER), biapenem (BIA), doripenem (DOR), ceftazidime (CAZ) and cefepime (FEP) against American MBL-producing isolates and also investigated the activity of ME1071 against the MBLs in cell-free extracts.

Methods: MICs of the drugs alone and in combination with 32 $\mu\text{g/ml}$ of ME1071 were determined by CLSI agar dilution methodology. The isolates comprised 14 MBL-producing *Pseudomonas aeruginosa* and one MBL-producing *Klebsiella pneumoniae*. The MBLs were IMP-7, IMP-18, VIM-2, VIM-7, two unidentified VIM enzymes, GIM-1, SPM-1, and an SPM enzyme. Spectrophotometric hydrolysis assays were performed using crude enzyme preparations and 100 μM carbapenem (CP) solutions.

Results: MICs of MER, DOR, BIA and CAZ were most reduced by ME1071. In general, MICs decreased 4-fold or more by ME1071, with an IMP-7 producing isolate exhibiting MIC reductions of at least 64-fold for IPM, BIA, and MER. The most active combination in terms of restoring susceptibility was CAZ + ME1071. Using a susceptible breakpoint of ≤ 4 $\mu\text{g/ml}$ for BIA, BIA + ME1071 was most effective CP-based combination. On the basis of IC_{50} values, ME1071 provided the greatest protection to BIA for which IC_{50} values were mostly < 30 μM .

Conclusions: In terms of MIC_{50} values, DOR, BIA, and CAZ were the most promising co-drugs for ME1071, while in terms of decreasing MICs to the susceptible range, CAZ + ME1071 was the most active combination. Until a larger collection of American isolates becomes available, the significance of these findings should be interpreted cautiously.

F1-1172

Quality Control Parameters for NXL104/ceftazidime Broth Microdilution Susceptibility Tests

M. M. TRACZEWSKI¹, S. D. BROWN ¹, P. LEVASSEUR ², C. MOISSEC ², Clinical Microbiology Institute, A. BRYSKIER ³; ¹Clinical Microbiol. Inst., Inc, Wilsonville, OR, ²Novoxel SA, Romainville, France, ³Novoxel, SA, Romainville, France.

Background: NXL104 is a new β -lactamase inhibitor which, when combined with ceftazidime, exhibits potent in vitro activity against a wide variety of β -lactamase producing microorganisms.

Methods: An eight-laboratory study was conducted to generate data to determine quality control (QC) limits for five standard QC organisms when testing susceptibility to NXL104/ceftazidime by the broth microdilution method. Three different lots of Mueller-Hinton broth were used. The broth was made up as either cation adjusted Mueller-Hinton Broth or *Haemophilus* Test Medium for testing *H. influenzae* and *K. pneumoniae*. All susceptibility tests were performed by methods outlined by the CLSI. Each laboratory performed 30 MIC determinations for each QC strain. NXL104 was maintained at a fixed concentration of 4 $\mu\text{g/ml}$ while the ceftazidime concentration varied over a range of 0.03 $\mu\text{g/ml}$ through 32 $\mu\text{g/ml}$.

Results: Colony counts ranged from 3.4×10^4 to 8.3×10^6 . Significant lot-to-lot variation was not observed. All tests were very reproducible and the following quality control limits are proposed (see table).

Conclusions: Microbroth dilution quality control ranges are proposed for NXL104/ceftazidime against 5 quality control strains recommended by the CLSI. These ranges have been proposed and accepted by the CLSI Subcommittee on Antimicrobial Susceptibility Testing.

Organism (ATCC)	Proposed Range ($\mu\text{g/ml}$)	% in Range
<i>E. coli</i> (25922)	0.06-0.5	99.6%
<i>S. aureus</i> (29213)	4-16	100%
<i>E. coli</i> (35218)	0.03-0.12	100%
<i>K. pneumoniae</i> (700603) in CAMHB	0.25 - 2	100%
<i>K. pneumoniae</i> (700603) in HTM	0.25 - 1	100%
<i>H. influenzae</i> (49247)	0.12 - 0.5	98.0%

L-662a

Superiority of an Antibiotic (faropenem medoxomil) versus Placebo in the Treatment of AECEB

R. M. ECHOLS¹, R. TOSIELLO¹, M. GARFIELD¹, K. WAY¹, A. RUTKOWSKI², S. KOWALSKY¹;

¹Replidyne, Inc., Louisville, CO, ²INC Res., Raleigh, NC.

Background: New FDA draft guidelines (August 2008) recommend only superiority trials for ABCEB-COPD studies. Replidyne has conducted a multinational placebo-controlled superiority trial in this patient population with faropenem medoxomil (FM).

Methods: Subjects had underlying COPD, defined by GOLD criteria class I - III; age ≥ 35 ; ≥ 10 pack years smoking; and acute worsening of cough, dyspnea, sputum production and purulence. Double blinded, randomized 2:1 to FM 600 mg or placebo BID X 5 days. Subjects evaluated for clinical response 3-7 days (test of cure) and 14-28 days post Rx. Alternative "rescue" antibiotics were permitted, but constituted failure in analysis. No boost in corticosteroids permitted. Primary analysis population (mITT) included subjects with AECEB, received study drug and had bacterial pathogen from sputum culture. Sample size estimate was 528 to achieve mITT of 264. Trial was terminated early for fiscal reasons. Safety monitoring included daily subject contact and an independent safety board.

Results: 491 subjects enrolled, 488 randomized, from 12 countries. COPD class: I=12%, II=52%, III=34%, IV=2%. mITT = 110 FM; 53 placebo. Clinical cure 80% vs. 64%, respectively; $p=0.0289$. ITT = 263 FM, 133 placebo. Cure 80% vs. 71%; $p=0.0484$. Frequent pathogens included *H. influenzae*, *S. aureus*, *M. catarrhalis*, *H. parainfluenzae*, *P. aeruginosa*, *K. pneumoniae*, *S. pneumoniae*. **Safety:** Adverse events (AEs) similar overall; more gastrointestinal AEs in FM group; more respiratory AEs in placebo group. Trend for more serious AEs and premature treatment discontinuation due to AEs among placebo group. No deaths reported.

Conclusions: First multinational placebo controlled trial in AECEB to demonstrate superiority of antibiotics. Frequency of adverse events numerically similar; greater worsening of respiratory symptoms in placebo group. Benefit-risk favors antibiotic treatment in selected patients with AECEB.