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In vitro evaluation of BAL9141, a novel parenteral cephalosporin active against oxacillin-resistant staphylococci

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Community-acquired and nosocomial infections caused by multidrug-resistant Gram-positive pathogens continue to increase in prevalence and have become a serious problem in many parts of the world. BAL9141 is a member of the class of parenteral pyrrolidinone-3-ylidenemethyl cephalosporins, and has a broad spectrum of activity. In the current study, BAL9141 was tested against a large number (*n* = 2263) of recent isolates from various international surveillance programmes including 1097 Gram-positive strains. Susceptibility to (S) and activity of (mg/L) to BAL9141, based on proposed breakpoints (MIC_{50}/MIC_{90}/% S) were as follows: methicillin-susceptible *Staphylococcus aureus* (0.5/0.5/100%), methicillin-resistant *S. aureus* (MRSA) (1/2/100%), methicillin-susceptible coagulase-negative staphylococci (CoNS) (0.12/0.25/100%), methicillin-resistant CoNS (MR-CoNS) (1/2/100%), *Streptococcus pneumoniae* (≤0.015/0.25/100%), viridans group streptococci (0.03/0.5/99%), β-haemolytic streptococci (≤0.015/≤0.015/100%), *Enterococcus faecalis* (0.5/16/90%), *Enterococcus faecium* (>32/>32/22%), *Haemophilus influenzae* (0.06/0.06/100%), *Moraxella catarrhalis* (0.06/0.5/100%), *Neisseria gonorrhoeae* (0.03/0.06/100%) and *Neisseria meningitidis* (≤0.002/0.004/100%). BAL9141 susceptibility at ≤4 mg/L (100% S) surpassed that of ceftriaxone (CRO; 1% S) and quinupristin/dalfopristin (Q-D; 92% S) against MRSA and MR-CoNS (CRO 0.9% S; Q-D 94% S). All *S. pneumoniae* were inhibited by BAL9141 at ≤1 mg/L compared with CRO (90% S) and levofloxacin (LVX; 98% S). Susceptibility rates for viridans group streptococci to BAL9141 (>98%) were also higher than to CRO (86%) and LVX (96%). BAL9141 demonstrated excellent activity against most species of wild-type enteric bacilli, with ≥95% of isolates being susceptible; however, only modest activity was observed for BAL9141 against non-fermentative Gram-negative species and ESBL-producing *Escherichia coli* or *Klebsiella pneumoniae*. BAL9141 demonstrated excellent activity against many tested pathogens displaying various resistance phenotypes, and should be particularly valuable in the treatment of MRSA as well as for drug-resistant streptococci, while maintaining a spectrum resembling a 'third-generation' cephalosporin against other clinically important species.

Keywords: BAL9141, parenteral cephalosporin, MRSA, antimicrobial activity, resistance

Introduction

The problem of antimicrobial resistance among Gram-positive cocci has become critical to effective therapy of these organisms in many parts of the world.[1–11] Various types of resistance continue to increase, whereas others, such as penicillin resistance in *Streptococcus pneumoniae*[7,8] or glycopeptide resistance among enterococci,[2,9,12] have stabilized at a worrisome elevated level. A number of factors have contributed to the emerging resistances, principally antimicrobial usage patterns (local, regional, national) and the quality of the infection control or public health infra-structures.[6] Even under better controls of formulary practice and hospital hygiene, the incidence of methicillin-resistant (MR) staphylococci in North American and European medical centres appears to be on the rise, as documented by the SENTRY

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Antimicrobial Surveillance Program. In the SENTRY Program, the MR *Staphylococcus aureus* (MRSA) rates between 1997 and 2000 increased in Europe (23–34%), North America (26–36%) and in the Asia–Pacific region (49–54%). Methicillin resistance among *S. aureus* or coagulase-negative staphylococci (CoNS) has been associated with high rates of co-resistance to fluoroquinolones such as ciprofloxacin and levofloxacin.[2,12] Therefore, continued use of broad-spectrum β-lactams (third- or fourth-generation cephalosporins, some penicillin/β-lactamase inhibitor combinations, carbapenems) and fluoroquinolones is likely to foster an environment conducive to increased occurrence of MR staphylococci as infecting pathogens or colonizing microflora. An attempt to develop drugs in both of these antimicrobial classes with significant activity versus MR staphylococci could facilitate a reduction in this clinical problem.

Recently marketed fluoroquinolones (gatifloxacin, moxifloxacin, trovafloxacin) have possessed enhanced activity and spectra against MR staphylococci, although the proportion of these isolates inhibited still falls below 90%.[13] In contrast, novel parenteral cephalosporins have not been widely studied or developed in recent years.[14,15] Among the recently described cephalosporins, the most studied has been RWJ-54428 (formerly MC-02,479).[16,17] This parenteral agent reportedly has MIC_{50} and MIC_{90} values for MRSA of 1 and 2 mg/L, respectively, with the highest observed MIC being only 4 mg/L.[10] Similar MIC results for MRSA have been reported for BMS-247243 (MIC_{90} 4 mg/L), S-3578 (MIC_{90} 4 mg/L) and SM-197436 (MIC_{90} 2 mg/L), all parenteral cephalosporin derivatives.[18–20] BAL9141 (formerly Ro63-9141) is a new member of the pyrrolidinone-3-ylidenemethyl cephems that has documented activity against MR staphylococci (MIC_{90} 2–4 mg/L), *Enterococcus faecalis* (MIC_{90} 4 mg/L) and penicillin-resistant pneumococci (MIC_{90} 2 mg/L), while preserving the anti-Gram-negative activity of third- or fourth-generation cephalosporins.[21,22] The mode of action of BAL9141 against staphylococci with altered PBP2a is a very high PBP enzyme affinity coupled with resistance to β-lactamases and a more stable acyl–enzyme complex.[21] Reports by the manufacturer of animal models of septicaemia, subcutaneous abscess and endocarditis showed that modest doses (10 mg/kg intraperitoneally) of BAL9141 effectively treated MR and vancomycin-intermediate *S. aureus* (VISA).[23] Andes & Craig[24] reported that BAL9141 exhibited *in vivo* cidal activity, with a moderate post-antibiotic effect (PAE; 3.8–4.8 h) against MRSA, and that $T > MIC$ was the pharmacokinetic/pharmacodynamic (PK/PD) parameter best predicting *in vitro* efficacy.

This investigation was designed to confirm and extend the earlier presentations by the manufacturer about the potency and spectrum of BAL9141. A worldwide sample of organisms was selected from recent resistance surveillance trials, and tests were of reference quality[25–27] against over 2200 isolates. Organism subsets were chosen to include major Gram-positive and -negative species, community-acquired respiratory pathogens, problematic endemic species causing meningitis or sexually transmitted disease, and difficult-to-treat anaerobic pathogens. Each category included isolates with resistance to different classes of drugs to challenge the spectrum of BAL9141 compared with numerous other antimicrobials.[28]

Materials and methods

Antimicrobials tested

The BAL9141 reagent grade compound was provided by Hoffmann-LaRoche AG and Basilea Pharmaceutica AG (Basle, Switzerland). Comparator agents were purchased from Sigma Chemical Co. (St Louis, MO, USA) or obtained from their respective manufacturers in the USA. A total of up to 13 comparators were evaluated depending upon the species tested. These compounds included β-lactams [penicillins, cephalosporins, penicillin/β-lactamase inhibitor combinations, a monobactam (aztreonam) and carbapenems], fluoroquinolones, aminoglycosides, trimethoprim/sulfamethoxazole and Gram-positive focused agents (macrolide-lincosamide-streptogramins, glycopeptides, oxazolidinones).

Organisms tested

The test strains (more than 2200) used in this study were derived from worldwide surveillance trials between 1997 and 2000. Larger numbers of resistant phenotypes were selected among the Gram-positive cocci to challenge the BAL9141 spectrum. The following organisms comprised the Gram-positive organism collection: 146 *S. aureus* (96 MRSA), 116 CoNS (90 MR-CoNS), 132 *Enterococcus* spp. (50 vancomycin resistant; *vanA*, *vanB*, *vanC* phenotypes), 520 *S. pneumoniae* (259 penicillin non-susceptible), 85 viridans group streptococci (31 penicillin non-susceptible) and 103 β-haemolytic streptococci (12 macrolide resistant). A subset of common Gram-negative respiratory tract pathogens included 415 strains of *Haemophilus influenzae* (155 ampicillin resistant, including 10 that were β-lactamase negative) and 188 isolates of *Moraxella catarrhalis* (167 penicillin resistant). Members of the Enterobacteriaceae included *Escherichia coli* [43 wild-type, 23 having an extended-spectrum β-lactamase (ESBL) phenotype], *Klebsiella pneumoniae* (30 wild-type, 25 ESBL phenotypes), *Klebsiella oxytoca* (12), *Enterobacter* spp. (95), *Citrobacter* spp. (52), *Salmonella* spp. (12), *Shigella* spp. (12), *Serratia* spp. (25), indole-positive *Proteae* (34) and *Proteus mirabilis* (nine). Non-fermentative species tested include *Pseudomonas aeruginosa* (23), *Acinetobacter* spp. (22), *Stenotrophomonas maltophilia* (17) and *Burkholderia cepacia* (eight). *Neisseria meningitidis* (24), *Neisseria gonorrhoeae* (32), *Bacteroides fragilis* group

(44) and *Clostridium* spp. (10) were also sampled. All strains were identified by at least two laboratories to species level. Strains were stored at –70°C or below until processed.

Susceptibility testing methods

All tests were carried out using the reference broth microdilution or agar dilution (*Neisseria* spp. and anaerobes) methods described by the NCCLS.[25,26] Cation-adjusted Mueller–Hinton broth was modified for streptococci by supplementation with 5% lysed horse blood, whereas for *H. influenzae* the Haemophilus Test Medium (HTM) formulation was used. Brucella blood agar and supplemented GC agar (IsoVitaleX) were used for anaerobes and pathogenic *Neisseria* spp., respectively. Isolates with ESBL phenotypes for *E. coli* and *K. pneumoniae* were selected using the NCCLS criteria[26] and all enzymes were shown to be inhibited by clavulanic acid (≥8-fold reduction in the MIC). All tests followed NCCLS technical details[25,27] for incubation temperature and environment, and incubation times before determining MIC endpoints. The quality control organisms used were: *E. coli* American Type Culture Collection (ATCC) 25922 and 35218, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *H. influenzae* ATCC 49247, *S. pneumoniae* ATCC 49619, *N. gonorrhoeae* ATCC 49226, *B. fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741.

Interpretative criteria were those published in NCCLS M100-S12[27] and a conservative ≤4 mg/L was used for BAL9141 (comparisons only) when testing enterococci, Enterobacteriaceae, non-enteric Gram-negative bacilli and staphylococci, whereas BAL9141 breakpoints for *Haemophilus* spp. and streptococci were set at ≤2 and ≤1 mg/L, respectively (those levels currently utilized for cefepime, cefotaxime and ceftriaxone).[27] Particular attention was made to accurately characterize staphylococci for oxacillin (methicillin) resistance[29,30] and to detect strains having reduced susceptibilities to glycopeptides.[3,4]

Results

BAL9141 activity against staphylococci

Table 1 presents the results of testing BAL9141 and 13 other agents against *S. aureus* and CoNS isolates (262 strains). All

Table 1. Antimicrobial activity of BAL9141 (formerly Ro63-9141) and 13 other comparison drugs tested against *Staphylococcus* and *Enterococcus* spp.

Organism (no. tested)	Antimicrobial agent	MIC (mg/L)			% Susceptible[a]
		50%	90%	range	
S. aureus					
oxacillin-susceptible (50)	BAL9141	0.5	0.5	0.25–2	(100.0)[b]
	ceftriaxone	4	4	2–8	100.0
	oxacillin	0.5	1	0.12–2	100.0
	penicillin	8	>32	≤0.015–>32	10.0
	co-amoxiclav	≤2	≤2	≤2–>16	96.0
	erythromycin	0.25	>8	0.25–>8	78.0
	clindamycin	0.12	0.12	≤0.06–0.25	100.0
	vancomycin	1	1	0.5–2	100.0
	teicoplanin	1	1	0.25–4	100.0
	linezolid	2	2	1–2	100.0
	quinupristin/dalfopristin	0.25	0.5	0.25–0.5	100.0
	ciprofloxacin	≤0.25	1	≤0.25–>2	98.0
	levofloxacin	0.25	0.25	0.06–4	98.0
	trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5	100.0
oxacillin-resistant (96)	BAL9141	1	2	0.12–2	(100.0)
	ceftriaxone	>32	>32	4–>32	1.0
	oxacillin	>8	>8	8–>8	0.0
	penicillin	32	>32	2–>32	0.0
	co-amoxiclav	>16	>16	≤2–>16	0.0
	erythromycin	>8	>8	0.25–>8	7.3
	clindamycin	>8	>8	≤0.06–>8	29.2
	vancomycin	1	2	0.5–4	100.0
	teicoplanin	1	4	0.25–>16	97.9
	linezolid	2	2	0.5–4	100.0

Table 1. (*Continued*)

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) 50%	90%	range	% Susceptible[a]
	quinupristin/dalfopristin	0.5	1	0.25–>8	91.7
	ciprofloxacin	>2	>2	≤0.25–>2	5.2
	levofloxacin	>4	>4	0.12–>4	7.2
	trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5–>2	75.0
Coag.-neg. staphylococci					
oxacillin-susceptible (26)	BAL9141	0.12	0.25	≤0.015–1	(100.0)
	ceftriaxone	1	8	≤0.25–8	100.0
	oxacillin	0.12	0.25	≤0.06–0.25	100.0
	penicillin	0.5	2	≤0.015–8	42.3
	co-amoxiclav	≤2	≤2	≤2	100.0
	erythromycin	0.25	>8	0.12–>8	73.1
	clindamycin	≤0.06	0.25	≤0.06–>8	96.2
	vancomycin	1	2	0.5–2	100.0
	teicoplanin	1	4	≤0.12–4	100.0
	linezolid	1	2	0.5–2	100.0
	quinupristin/dalfopristin	0.25	0.5	0.12–0.5	100.0
	ciprofloxacin	≤0.25	≤0.25	≤0.25–>2	92.3
	levofloxacin	0.12	0.25	0.12–>4	92.3
	trimethoprim/sulfamethoxazole	≤0.5	>1	≤0.5–>1	73.1
oxacillin-resistant (90)	BAL9141	1	2	≤0.015–4	(100.0)
	ceftriaxone	16	>32	2–>32	0.0
	oxacillin	>8	>8	0.5–>8	0.0
	penicillin	8	32	0.06–>32	0.0
	co-amoxiclav	≤2	>16	≤2–>16	0.0
	erythromycin	>8	>8	0.12–>8	4.4
	clindamycin	>8	>8	≤0.06–>8	37.8
	vancomycin	2	2	1–8	98.9
	teicoplanin	4	16	0.25–>16	86.7
	linezolid	1	2	0.25–2	100.0
	quinupristin/dalfopristin	0.25	1	0.12–>8	94.4
	ciprofloxacin	>2	>2	≤0.25–>2	26.7
	levofloxacin	4	>4	0.12–>4	34.8
	trimethoprim/sulfamethoxazole	>1	>1	≤0.5–>1	33.3
E. faecalis (62)[c]	BAL9141	0.5	4	0.12–>32	(90.3)
	ceftriaxone	>32	>32	>32	–
	ampicillin	2	4	1–>16	93.5
	penicillin	4	8	1–>32	90.3
	co-amoxiclav	≤2	4	≤2–>16	93.5
	imipenem	2	8	0.5–>8	–
	erythromycin	>8	>8	0.25–>8	11.3
	vancomycin	2	>16	0.5–>16	67.7
	teicoplanin	0.25	>16	≤0.12–>16	83.9
	linezolid	2	2	0.5–2	100.0
	quinupristin/dalfopristin	8	>8	1–>8	6.5
	ciprofloxacin	>2	>2	0.5–>2	40.3
	levofloxacin	2	>4	1–>4	52.8
	trimethoprim/sulfamethoxazole	≤0.5	>1	≤0.5–>1	–
Enterococcus faecium (51)[d]	BAL9141	>32	>32	0.25–>32	(21.6)
	ceftriaxone	>32	>32	2–>32	–
	ampicillin	>16	>16	≤0.12–>16	25.5
	penicillin	>32	>32	0.25–>32	19.6
	co-amoxiclav	>16	>16	≤2–>16	25.5

Table 1. (*Continued*)

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) 50%	90%	range	% Susceptible[a]
	imipenem	>8	>8	0.12–>8	–
	erythromycin	>8	>8	0.12–>8	9.8
	vancomycin	2	>16	≤0.12–>16	52.9
	teicoplanin	1	>16	≤0.12–>16	52.9
	linezolid	2	2	≤0.06–2	100.0
	quinupristin/dalfopristin	0.5	>8	0.25–>8	66.7
	ciprofloxacin	>2	>2	0.5–>2	11.8
	levofloxacin	2	>4	0.5–>4	56.0
	trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5–>2	–
Enterococcus spp. (19)[e]	BAL9141	0.5	>32	0.12–>32	(73.7)
	ceftriaxone	>32	>32	32–>32	–
	ampicillin	1	>16	0.5–>16	73.7
	penicillin	2	>32	0.5–>32	68.4
	co-amoxiclav	≤2	>16	≤2–>16	73.7
	imipenem	2	>8	0.5–>8	–
	erythromycin	2	>8	≤0.06–>8	31.6
	vancomycin	2	8	0.5–16	68.4
	teicoplanin	0.5	1	≤0.12–>16	94.7
	linezolid	2	2	1–2	100.0
	quinupristin/dalfopristin	2	8	0.25–>8	10.5
	ciprofloxacin	2	>2	0.5–>2	36.8
	levofloxacin	2	>4	1–>4	68.4
	trimethoprim/sulfamethoxazole	≤0.5	>1	≤0.5–>1	–

[a]MIC interpretation criteria of the NCCLS.[27]
[b]A breakpoint of ≤4 mg/L was used for BAL9141 for comparisons only (% in parentheses).
[c]Twenty vancomycin-resistant strains were tested, one-half *vanA* and the remainder *vanB*.
[d]Includes 24 vancomycin-resistant strains (20 *vanA*, four *vanB*).
[e]Includes five species groups, six strains with *vanC* phenotypes.

BAL9141 MIC values were ≤2 mg/L for *S. aureus*, with MIC_{50} and MIC_{90} values of 0.5 and 0.5 mg/L and 1 and 2 mg/L for oxacillin-susceptible (OS) and MR (also oxacillin-resistant) strains, respectively. All antimicrobials except penicillin were effective *in vitro* against OS *S. aureus*, with susceptibility ranging from 78.0% (erythromycin) to 100.0% (eight agents). However, for the 96 strains of MRSA, only BAL9141, vancomycin (MIC_{90} 2 mg/L; 100.0% susceptible) and linezolid (MIC_{90} 2 mg/L; 100.0% susceptible) remained highly effective. Methicillin-sensitive strains were slightly more susceptible (two- to four-fold) to BAL9141 than MRSA strains.

CoNS strains were slightly more susceptible to BAL9141 than *S. aureus* isolates, although the highest BAL9141 MIC was identified for an MR-CoNS isolate. OS-CoNS strains were more susceptible to BAL9141 when compared with MR-CoNS isolates. All antimicrobials tested had a ≥92.3% susceptibility rate versus OS-CoNS, except penicillin (42.3% susceptible), erythromycin (73.1%) and trimethoprim/sulfamethoxazole (73.1%). Only four tested antimicrobials inhibited MR-CoNS strains at a rate >90%: BAL9141 (100.0% inhibited at ≤4 mg/L), linezolid (100.0% susceptible), vancomycin (98.9% susceptible) and quinupristin/dalfopristin (94.4% susceptible). All CoNS strains selected to challenge BAL9141 against defined glycopeptide-intermediate (one strain), macrolide and clindamycin-resistant (56 strains), streptogramin-resistant (five strains) and fluoroquinolone-resistant (59 strains) populations were inhibited by ≤4 mg/L BAL9141.

BAL9141 activity against enterococci

Table 1 also lists BAL9141 activity tested against 132 strains of enterococci. The BAL9141 activity versus *E. faecalis* paralleled that of ampicillin (93.5% susceptible), penicillin (90.3%) and co-amoxiclav (93.5%). The BAL9141 MIC_{90} (4 mg/L) was identical to that of ampicillin and co-amoxiclav. Ciprofloxacin and levofloxacin were active against 40.3–52.8% of strains at MICs at or below their NCCLS breakpoints.

E. faecium ($n = 51$) strains, nearly one-half with the *vanA* resistance pattern, ranged from 19.6% to 25.5% susceptible to tested penicillin derivatives. BAL9141 inhibited 21.6% of *E. faecium* strains at ≤4 mg/L. All strains were linezolid susceptible. Quinupristin/dalfopristin inhibited 66.7% of *E. faecium* at ≤1 mg/L, with many isolates possessing an MIC of 2 mg/L (intermediate). The remaining *Enterococcus* spp. tested from five species (six *vanC*) demonstrated that BAL9141 activity was most like that of the tested penicillins (MIC_{50} range 0.5–2 mg/L). Overall, linezolid had the greatest potency against all enterococci tested, a collection enhanced by large numbers of strains with defined resistance mechanisms.

BAL9141 activity against streptococci

Table 2 shows the potency of BAL9141 and 12 comparison agents tested against various streptococcal species groups listed according to their susceptibilities to penicillin. BAL9141 activity was at least four-fold greater than that of ceftriaxone towards strains with reduced susceptibilities to penicillin (MIC ≥ 0.12 mg/L). Among the four clinically available β-lactams tested, ceftriaxone and amoxicillin ± clavulanate demonstrated nearly complete coverage of pneumococci with penicillin MICs of ≤1 mg/L. However, only 52.6–58.8% of penicillin-resistant strains were inhibited by these agents. Generally, the fluoroquinolones tended to be more active than the β-lactams.

Activity of the β-lactams (including BAL9141), macrolides, clindamycin and trimethoprim/sulfamethoxazole decreased as the penicillin MIC increased. The potency of BAL9141 was equal to that of the fluoroquinolones against penicillin-resistant *S. pneumoniae* on a weight–weight basis (Table 2).

Viridans group streptococci were generally more resistant to the β-lactams, macrolides, clindamycin, fluoroquinolones and trimethoprim/sulfamethoxazole than the *S. pneumoniae* isolates. BAL9141 MIC_{90} results varied from 0.06 mg/L for penicillin-susceptible strains to 0.25 and 1 mg/L for penicillin-intermediate and -resistant strains, respectively. At current NCCLS breakpoints for susceptibility, ceftriaxone and co-amoxiclav were most potent among the β-lactams, but the glycopeptides (vancomycin, teicoplanin) and quinupristin/dalfopristin had the highest level of susceptibility (≥90.0%). If the current breakpoint for ceftriaxone (susceptible at ≤1 mg/L) was applied to BAL9141, only one viridans group streptococcus strain would *not* have been judged BAL9141 susceptible (1.2%). β-Haemolytic streptococci were very susceptible to BAL9141 ($MIC_{90} \leq 0.015$ mg/L) and most other drugs tested. Only erythromycin had a susceptibility rate <98.0% (88.3%).

BAL9141 activity against H. influenzae and M. catarrhalis

H. influenzae strains were uniformly susceptible to BAL9141 (MIC_{90} 0.06 mg/L), as well as to ceftriaxone and cefepime (Table 3). No changes in the MIC_{50} and MIC_{90} (0.06 mg/L) values of BAL9141 were observed for strains with a β-lactamase-positive phenotype (data not shown). β-Lactamase-negative ampicillin-resistant (BLNAR) *H. influenzae* isolates ($n = 10$) showed four- to eight-fold elevated BAL9141 MIC values (MIC_{50} 0.25 mg/L, MIC_{90} 0.5 mg/L) compared with the remaining 405 strains tested. All fluoroquinolone non-susceptible strains tested were inhibited by ≤0.06 mg/L BAL9141. The least active antimicrobials tested were ampicillin (145 β-lactamase-positive strains; 64.2% susceptible), clarithromycin (82.8% susceptible) and trimethoprim/sulfamethoxazole (77.8% susceptible).

M. catarrhalis isolates lacking β-lactamase production (11.2%) were significantly more susceptible (MIC_{90} 0.015 mg/L) to BAL9141 compared with their β-lactamase-positive counterparts (MIC_{90} 0.5 mg/L; data not shown). Only three isolates among 188 strains tested (1.6%) had a BAL9141 MIC of 1 mg/L (Table 3). Overall, *M. catarrhalis* isolates were very susceptible to all β-lactams tested (≥95.2% susceptible) with the exception of penicillin.

BAL9141 activity against Enterobacteriaceae

BAL9141 exhibited good activity against Gram-negative enteric bacilli with susceptibility rates that ranged between 79.4% and 100% using a proposed breakpoint of ≤4 mg/L (Table 4). Significant BAL9141 potency was most notable for *Citrobacter* spp., *E. coli*, *Enterobacter cloacae*, *Klebsiella* spp., *P. mirabilis*, *Salmonella* spp. and *Shigella* spp., which collectively had MIC_{90} values ranging from 0.03 to 0.5 mg/L. Against *Citrobacter freundii* the MIC_{90} of BAL9141 (0.5 mg/L) was the same as noted for cefepime and imipenem, with susceptibility rates also similar for these compounds and the two fluoroquinolones tested (>94%). The remaining effective compounds *in vitro* showed a rank order of: gentamicin (94.1%) > piperacillin/tazobactam, aztreonam (85.3%) > ceftazidime, ceftriaxone (82.4%). The *E. coli* isolates were all susceptible to BAL9141, cefepime, and the carbapenems with very similar potencies ($MIC_{90} \leq 0.12$–0.25 mg/L). The extended- and broad-spectrum cephalosporins piperacillin/tazobactam, gentamicin and aztreonam were slightly less active (95.3–97.7% susceptible). The two fluoroquinolones showed equivalent activities (86% susceptible), but a rate similar only to that of cefazolin. *E. cloacae* isolates were most susceptible to cefepime and the carbapenems (100%); however, the potency of BAL9141 (MIC_{90} 0.12 mg/L) was equal to or greater than these compounds, and shared an activity most similar to gentamicin and the fluoroquinolones (98.3% susceptible). Against *K. pneumoniae*, all test agents showed excellent activity, with BAL9141 activity most similar to that of cefoxitin and ciprofloxacin (96% susceptible). All compounds were active against *K. oxytoca* (except cefazolin; only 75% susceptible), *P. mirabilis*, *Salmonella* spp. (except quinolones; only 90.9% susceptible) and

Table 2. Antimicrobial activity of BAL9141 (formerly Ro63-9141) and 12 other comparison drugs tested against *Streptococcus* spp.

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) 50%	90%	range	% Susceptible[a]
S. pneumoniae					
penicillin-S (261)	BAL9141	≤0.015	≤0.015	≤0.015–0.03	(100.0)[b]
	ceftriaxone	0.015	0.06	≤0.008–0.5	100.0
	amoxicillin	≤0.06	≤0.06	≤0.06–0.5	100.0
	penicillin	≤0.03	0.06	≤0.03–0.06	100.0
	co-amoxiclav	≤0.25	≤0.25	≤0.25	100.0
	erythromycin	≤0.25	2	≤0.25–>32	88.9
	clindamycin	≤0.25	≤0.25	≤0.25–>2	94.3
	vancomycin	0.25	0.5	≤0.12–1	100.0
	quinupristin/dalfopristin	≤0.25	0.5	≤0.25–1	100.0
	ciprofloxacin	1	2	0.03–>2	–
	levofloxacin	1	1	≤0.03–>4	97.3
	moxifloxacin	0.12	0.25	≤0.03–>4	97.3
	trimethoprim/sulfamethoxazole	0.5	1	≤0.5–>4	85.4
penicillin-I (145)	BAL9141	0.06	0.12	≤0.015–0.5	(100.0)
	ceftriaxone	0.25	1	≤0.008–8	97.2
	amoxicillin	0.5	1	≤0.06–4	99.3
	penicillin	0.5	1	0.12–1	0.0
	co-amoxiclav	≤0.25	1	≤0.25–4	99.3
	erythromycin	1	>32	≤0.25–>32	44.9
	clindamycin	≤0.25	>2	≤0.25–>2	75.9
	vancomycin	0.5	0.5	≤0.12–1	100.0
	quinupristin/dalfopristin	0.5	0.5	≤0.25–1	100.0
	ciprofloxacin	1	2	≤0.015–>2	–
	levofloxacin	1	1	≤0.03–1	100.0
	moxifloxacin	0.12	0.12	≤0.03–0.25	100.0
	trimethoprim/sulfamethoxazole	1	>4	≤0.5–>4	46.5
penicillin-R (114)	BAL9141	0.25	0.25	≤0.015–1	(100.0)
	ceftriaxone	1	4	0.5–8	58.8
	amoxicillin	2	8	1–>8	52.6
	penicillin	4	4	2–>4	0.0
	co-amoxiclav	2	8	1–>8	52.6
	erythromycin	4	>32	≤0.25–>32	22.8
	clindamycin	≤0.25	>2	≤0.25–>2	65.8
	vancomycin	0.5	0.5	≤0.12–1	100.0
	quinupristin/dalfopristin	0.5	1	≤0.25–1	100.0
	ciprofloxacin	1	2	0.5–>2	–
	levofloxacin	1	1	0.12–>4	96.5
	moxifloxacin	0.12	0.25	0.06–4	96.5
	trimethoprim/sulfamethoxazole	4	>4	≤0.5–>4	7.9
Viridans group streptococci					
penicillin-S (54)	BAL9141	≤0.015	0.06	≤0.015–0.06	(100.0)
	ceftriaxone	≤0.25	≤0.25	≤0.25–0.5	100.0
	ampicillin	≤0.12	≤0.12	≤0.12–1	100.0
	penicillin	0.03	0.12	≤0.015–0.12	100.0
	co-amoxiclav	≤2	≤2	≤2	100.0
	erythromycin	≤0.06	4	≤0.06–>8	55.6
	clindamycin	≤0.06	0.12	≤0.06–>8	90.7
	vancomycin	0.5	1	0.25–1	100.0
	teicoplanin	≤0.12	≤0.12	≤0.12	100.0
	quinupristin/dalfopristin	0.5	1	0.12–2	98.1

Table 2. (*Continued*)

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) 50%	90%	range	% Susceptible[a]
	ciprofloxacin	2	>2	≤0.25–>2	–
	levofloxacin	1	1	0.25–>4	98.1
	trimethoprim/sulfamethoxazole	≤0.5	1	≤0.5–>1	75.9
penicillin-I (21)	BAL9141	0.03	0.25	≤0.015–1	(100.0)
	ceftriaxone	≤0.25	1	≤0.25–2	85.7
	ampicillin	0.5	2	≤0.12–2	0.0
	penicillin	0.25	1	0.25–1	0.0
	co-amoxiclav	≤2	≤2	≤2–4	95.2
	erythromycin		4	≤0.06–>8	57.1
	clindamycin	≤0.06	≤0.06	≤0.06–>8	90.5
	vancomycin	0.5	1	0.25–1	100.0
	teicoplanin	≤0.12	≤0.12	≤0.12–0.25	100.0
	quinupristin/dalfopristin		1	0.25–2	95.2
	ciprofloxacin	2	>2	0.5–>2	–
	levofloxacin	1	1	0.5–>4	90.5
	trimethoprim/sulfamethoxazole	≤0.5	>1	≤0.5–>1	66.7
penicillin-R (10)	BAL9141	0.5	1	0.12–32	(90.0)
	ceftriaxone	4	8	2–>32	0.0
	ampicillin	8	>16	8–>16	0.0
	penicillin	4	8	4–>32	0.0
	co-amoxiclav	8	16	4–>16	0.0
	erythromycin	1	8	0.5–>8	10.0
	clindamycin	≤0.06	≤0.06	≤0.06–>8	90.0
	vancomycin	0.5	1	0.25–1	100.0
	teicoplanin	≤0.12	≤0.12	≤0.12	100.0
	quinupristin/dalfopristin	0.5	1	0.5–2	90.0
	ciprofloxacin	2	>2	1–>2	–
	levofloxacin	1	2	0.5–2	100.0
	trimethoprim/sulfamethoxazole	>1	>1	1–>1	0.0
β-Haemolytic streptococci (103)[c]	BAL9141	≤0.015	≤0.015	≤0.015–0.06	(100.0)
	ceftriaxone	≤0.25	≤0.25	≤0.25	100.0
	ampicillin	≤0.12	≤0.12	≤0.12	100.0
	penicillin	≤0.015	0.06	≤0.015–0.06	100.0
	co-amoxiclav	≤2	≤2	≤2	100.0
	erythromycin	≤0.06	1	≤0.06–>8	88.3
	clindamycin	≤0.06	≤0.06	≤0.06–>8	98.0
	vancomycin	0.5	0.5	0.12–1	100.0
	teicoplanin	≤0.12	≤0.12	≤0.12–0.25	100.0
	quinupristin/dalfopristin	0.25	0.5	≤0.06–1	100.0
	ciprofloxacin	0.5	1	≤0.25–>2	–
	levofloxacin	0.5	1	0.25–>4	99.0
	trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5	100.0

[a]NCCLS[27] breakpoint criteria were used.
[b]For BAL9141, those susceptible breakpoints listed by the NCCLS[27] for ceftriaxone were applied as indicated for non-meningitis infections (% susceptible in parentheses).
[c]Includes 48 group A, 36 group B, eight group C, one group F and 10 group G isolates.

Shigella spp. (except penicillin/β-lactam inhibitor combinations; only 83.3–91.7% susceptible).

Some isolates of *Enterobacter aerogenes*, *Serratia* spp. and certain species of indole-positive *Proteae* showed more limited BAL9141 inhibition. Against *E. aerogenes* the rank order of activity was: carbapenems (100% susceptible) > cefepime > gentamicin > fluoroquinolones > BAL9141, ceftriaxone (83.8% and 81.1%), with the remaining com-

Table 3. Antimicrobial activity of BAL9141 (formerly Ro63-9141) and 13 other comparison drugs tested against commonly isolated Gram-negative respiratory tract pathogens

Organism (no. tested)	Antimicrobial agent	MIC (mg/L)			% Susceptible[a]
		50%	90%	range	
H. influenzae (405)[c]	BAL9141	0.06	0.06	≤0.015–0.25	(100.0)[b]
	ceftriaxone	≤0.008	0.03	≤0.008–0.03	100.0
	ampicillin	≤0.5	8	≤0.5–>16	64.2
	cefepime	≤0.12	≤0.12	≤0.12–0.5	100.0
	cefuroxime	1	2	≤0.06–>8	98.8
	co-amoxiclav	0.5	2	≤0.25–8	99.3
	piperacillin/tazobactam	≤0.06	≤0.06	≤0.06–0.25	100.0
	erythromycin	4	8	≤0.25–>8	–
	azithromycin	1	2	≤0.12–16	99.4
	clarithromycin	8	16	≤0.25–>32	82.8
	ciprofloxacin	≤0.015	1	≤0.015–2	99.8
	levofloxacin	≤0.03	≤0.03	≤0.03–1	100.0
	moxifloxacin	≤0.03	≤0.03	≤0.03–0.12	100.0
	trimethoprim/sulfamethoxazole	≤0.5	>4	≤0.5–>4	77.8
H. influenzae (10)[d]	BAL9141	0.25	0.5	0.12–0.25	(100.0)
M. catarrhalis (188)[e]	BAL9141	0.06	0.5	≤0.015–1	(100.0)
	ceftriaxone	0.12	0.5	≤0.008–1	100.0
	penicillin	4	>4	≤0.03–>4	11.2
	cefepime	0.5	2	≤0.06–4	98.9
	cefuroxime	1	2	0.12–4	100.0
	co-amoxiclav	≤0.25	≤0.25	≤0.25–0.5	100.0
	piperacillin/tazobactam	≤0.06	≤0.06	≤0.06–0.12	100.0
	erythromycin	≤0.25	≤0.25	≤0.25–4	–
	azithromycin	≤0.12	≤0.12	≤0.12–1	100.0
	clarithromycin	≤0.25	≤0.25	≤0.25–4	100.0
	ciprofloxacin	0.03	0.06	≤0.015–0.06	100.0
	levofloxacin	≤0.03	≤0.03	≤0.03–0.06	100.0
	moxifloxacin	0.06	0.06	≤0.03–0.12	100.0
	trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5–4	95.2

[a]Breakpoints for susceptibility published for *H. influenzae* by the NCCLS[27] were applied.
[b]For BAL9141, the percentage in parentheses indicates that portion of strains with MIC ≤ 2 mg/L, for comparisons only. –, no criteria recognized by the NCCLS.[27]
[c]Includes 145 β-lactamase producers.
[d]β-Lactamase-negative, ampicillin-resistant (MIC ≥ 4 mg/L) strains.
[e]Includes 21 β-lactamase-negative strains with penicillin MICs ≤ 0.06 mg/L.

pounds having marginal or no activity. Among the indole-positive *Proteae*, *P. vulgaris* (one-third of the isolates in this group) was refractory to BAL9141 (MIC_{50}/MIC_{90}, % susceptible were 32/>32 mg/L, 36.4%). Regardless, the activity of BAL9141 (79.4%) towards these cited species remained higher than that of the comparator fluoroquinolones (76.9% susceptible), but lower than that of other 'broad-spectrum' cephalosporins, piperacillin/tazobactam, gentamicin, aztreonam and the carbapenems (88.5–100% susceptible).

Table 5 provides *in vitro* susceptibility data on *E. coli* and *K. pneumoniae* exhibiting confirmed ESBL phenotypes. With the exception of the carbapenems, the potency of all agents tested was diminished compared with wild-type ESBL-negative strains (Table 4). Indeed nearly all MIC_{90}s exceeded the maximum test concentrations, except for the carbapenems, which retained complete activity against the ESBL-positive strains. Among the *E. coli* isolates, BAL9141 activity was most similar to that of ceftriaxone and aztreonam, which in turn were more active than tetracycline and trimethoprim/sulfamethoxazole.

BAL9141 activity against non-fermentative Gram-negative bacilli

BAL9141 demonstrated widely variable activity against the tested non-fermentative Gram-negative bacilli, as shown by

Table 4. Antimicrobial activity of BAL9141 (formerly Ro63-9141) and 13 other comparison drugs tested against species of Enterobacteriaceae

Organism (no. tested)	Antimicrobial agent	MIC (mg/L)			% Susceptible[a]
		50%	90%	range	
C. freundii (34)	BAL9141	0.03	0.5	≤0.015–>32	(97.1)[b]
	cefazolin	>16	>16	≤2–>16	15.6
	cefoxitin	>32	>32	2–>32	14.7
	ceftazidime	≤2	>16	≤2–>16	82.4
	ceftriaxone	≤0.25	16	≤0.25–>32	82.4
	cefepime	≤0.12	0.5	≤0.12–>16	97.1
	co-amoxiclav	>16	>16	≤2–>16	17.6
	piperacillin/tazobactam	2	128	0.25–>128	85.3
	aztreonam	≤0.12	16	≤0.12–>16	85.3
	imipenem	0.25	0.5	≤0.06–1	100.0
	meropenem	≤0.06	≤0.06	≤0.06–0.12	100.0
	gentamicin	≤1	≤1	≤1–>8	94.1
	ciprofloxacin	≤0.25	≤0.25	≤0.015–2	97.1
	levofloxacin	≤0.03	0.25	≤0.03–2	100.0
Citrobacter koseri (18)	BAL9141	0.03	0.06	≤0.015–0.06	(100.0)
	cefazolin	≤2	≤2	≤2–4	100.0
	cefoxitin	2	4	1–4	100.0
	ceftazidime	≤2	≤2	≤2	100.0
	ceftriaxone	≤0.25	≤0.25	≤0.25	100.0
	cefepime	≤0.12	≤0.12	≤0.12	100.0
	co-amoxiclav	≤2	4	≤2–4	100.0
	piperacillin/tazobactam	2	8	1–16	100.0
	aztreonam	≤0.12	≤0.12	≤0.12	100.0
	imipenem	0.12	0.25	≤0.06–0.25	100.0
	meropenem	≤0.06	≤0.06	≤0.06	100.0
	gentamicin	≤1	≤1	≤1	100.0
	ciprofloxacin	≤0.25	≤0.25	≤0.25	100.0
	levofloxacin	≤0.03	0.25	≤0.03–0.25	100.0
E. coli (43)	BAL9141	0.03	0.06	≤0.015–2	(100.0)
	cefazolin	≤2	16	≤2–>16	85.7
	cefoxitin	2	4	2–>32	97.7
	ceftazidime	≤0.12	0.25	≤0.12–>16	95.3
	ceftriaxone	≤0.25	≤0.25	≤0.25–>32	95.3
	cefepime	≤0.12	≤0.12	≤0.12–4	100.0
	co-amoxiclav	4	16	≤2–>16	79.1
	piperacillin/tazobactam	2	4	0.5–128	95.3
	aztreonam	≤0.12	≤0.12	≤0.12–>16	95.3
	imipenem	0.12	0.25	≤0.06–0.25	100.0
	meropenem	≤0.06	≤0.06	≤0.06	100.0
	gentamicin	≤1	≤1	≤1–>8	97.7
	ciprofloxacin	≤0.25	>2	≤0.25–>2	86.0
	levofloxacin	≤0.03	4	≤0.03–>4	86.0
E. aerogenes (37)	BAL9141	0.03	>32	≤0.015–>32	(83.8)
	cefazolin	>16	>16	≤2–>16	20.0
	cefoxitin	>32	>32	2–>32	2.7
	ceftazidime	≤2	>16	≤2–>16	75.7
	ceftriaxone	≤0.25	>32	≤0.25–>32	81.1
	cefepime	≤0.12	4	≤0.12–>16	97.3
	co-amoxiclav	>16	>16	4–>16	2.7
	piperacillin/tazobactam	2	128	0.5–>128	73.0
	aztreonam	≤0.12	>16	≤0.12–>16	78.4
	imipenem	0.5	1	0.12–2	100.0

Table 4. (*Continued*)

Organism (no. tested)	Antimicrobial agent	MIC (mg/L)			% Susceptible[a]
		50%	90%	range	
	meropenem	≤0.06	0.12	≤0.06–2	100.0
	gentamicin	≤1	4	≤1–>8	91.9
	ciprofloxacin	≤0.25	>2	≤0.25–>2	89.2
	levofloxacin	≤0.03	>4	≤0.03–>4	89.2
E. cloacae (58)	BAL9141	0.06	0.12	0.03–>32	(98.3)
	cefazolin	>16	>16	≤2–>16	8.6
	cefoxitin	>32	>32	4–>32	3.4
	ceftazidime	≤2	≤2	≤2–>16	96.6
	ceftriaxone	≤0.25	0.5	≤0.25–>32	96.6
	cefepime	≤0.12	0.25	≤0.12–8	100.0
	co-amoxiclav	>16	>16	4–>16	5.2
	piperacillin/tazobactam	2	4	0.25–128	96.6
	aztreonam	≤0.12	0.5	≤0.12–>16	96.6
	imipenem	0.25	0.5	0.12–1	100.0
	meropenem	≤0.06	≤0.06	≤0.06–0.25	100.0
	gentamicin	≤1	≤1	≤1–>8	98.3
	ciprofloxacin	≤0.25	≤0.25	≤0.25–>2	98.3
	levofloxacin	≤0.03	0.25	≤0.03–>4	98.3
K. oxytoca (12)	BAL9141	0.06	0.5	≤0.015–0.5	(100.0)
	cefazolin	4	16	≤2–16	75.0
	cefoxitin	2	4	1–4	100.0
	ceftazidime	≤2	≤2	≤2	100.0
	ceftriaxone	≤0.25	≤0.25	≤0.25	100.0
	cefepime	≤0.12	≤0.12	≤0.12	100.0
	co-amoxiclav	≤2	≤2	≤2–4	100.0
	piperacillin/tazobactam	1	2	1–2	100.0
	aztreonam	≤0.12	≤0.12	≤0.12	100.0
	imipenem	0.12	0.25	0.12–0.25	100.0
	meropenem	≤0.06	≤0.06	≤0.06	100.0
	gentamicin	≤1	≤1	≤1	100.0
	ciprofloxacin	≤0.25	≤0.25	≤0.25	100.0
	levofloxacin	≤0.03	0.06	≤0.03–0.06	100.0
K. pneumoniae (30)	BAL9141	0.03	0.06	≤0.015–16	(96.7)
	cefazolin	≤2	≤2	≤2–>16	92.3
	cefoxitin	4	8	1–16	96.0
	ceftazidime	≤2	≤2	≤2	100.0
	ceftriaxone	≤0.25	≤0.25	≤0.25–2	100.0
	cefepime	≤0.12	≤0.12	≤0.12–0.5	100.0
	co-amoxiclav	≤2	8	≤2–>16	92.0
	piperacillin/tazobactam	2	16	1–64	90.0
	aztreonam	≤0.12	≤0.12	≤0.12–0.5	100.0
	imipenem	0.12	0.5	0.12–0.5	100.0
	meropenem	≤0.06	≤0.06	≤0.06–0.12	100.0
	gentamicin	≤1	≤1	≤1	100.0
	ciprofloxacin	≤0.25	≤0.25	≤0.25–2	96.0
	levofloxacin	≤0.03	0.25	≤0.03–2	100.0
P. mirabilis (9)	BAL9141	≤0.015	–	≤0.015–0.03	(100.0)
	cefazolin	4	–	4	100.0
	cefoxitin	2	–	2–4	100.0
	ceftazidime	≤2	–	≤2	100.0
	ceftriaxone	≤0.25	–	≤0.25	100.0
	cefepime	≤0.12	–	≤0.12–0.25	100.0
	co-amoxiclav	≤2	–	≤2	100.0

Table 4. (*Continued*)

Organism (no. tested)	Antimicrobial agent	MIC (mg/L)			% Susceptible[a]
		50%	90%	range	
	piperacillin/tazobactam	0.25	–	0.12–0.5	100.0
	aztreonam	≤0.12	–	≤0.12	100.0
	imipenem	1	–	0.25–2	100.0
	meropenem	≤0.06	–	≤0.06–0.12	100.0
	gentamicin	≤1	–	≤1–2	100.0
	ciprofloxacin	≤0.25	–	≤0.25	100.0
	levofloxacin	≤0.03	–	≤0.03–0.06	100.0
Indole-positive *Proteae* (34)[c]	BAL9141	≤0.015	>32	≤0.015–>32	(79.4)
	cefazolin	>16	>16	≤2–>16	8.0
	cefoxitin	4	16	1–>32	84.6
	ceftazidime	≤2	4	≤2–>16	92.3
	ceftriaxone	≤0.25	8	≤0.25–8	100.0
	cefepime	≤0.12	0.25	≤0.12–1	100.0
	co-amoxiclav	>16	>16	≤2–>16	30.8
	piperacillin/tazobactam	0.25	4	≤0.06–8	100.0
	aztreonam	≤0.12	0.5	≤0.12–1	100.0
	imipenem	1	2	0.12–2	100.0
	meropenem	≤0.06	0.12	≤0.06–0.25	100.0
	gentamicin	≤1	8	≤1–8	88.5
	ciprofloxacin	0.03	>2	≤0.015–>2	76.9
	levofloxacin	≤0.03	>4	≤0.03–>4	76.9
Salmonella spp. (12)	BAL9141	0.03	0.03	0.03–0.06	(100.0)
	cefazolin	≤2	4	≤2–4	100.0
	cefoxitin	2	4	1–4	100.0
	ceftazidime	≤2	≤2	≤2	100.0
	ceftriaxone	≤0.25	≤0.25	≤0.25–8	100.0
	cefepime	≤0.12	≤0.12	≤0.12–4	100.0
	co-amoxiclav	≤2	8	≤2–8	100.0
	piperacillin/tazobactam	2	4	1–16	100.0
	aztreonam	≤0.12	≤0.12	≤0.12	100.0
	imipenem	0.25	0.25	0.12–0.25	100.0
	meropenem	≤0.06	≤0.06	≤0.06	100.0
	gentamicin	≤1	≤1	≤1	100.0
	ciprofloxacin	≤0.015	≤0.015	≤0.015–>2	90.9
	levofloxacin	≤0.03	≤0.03	≤0.03–>4	90.9
Serratia spp. (25)	BAL9141	0.06	8	0.03–>32	(88.0)
	cefazolin	>16	>16	>16	0.0
	cefoxitin	16	>32	4–>32	23.8
	ceftazidime	≤2	8	≤2–16	95.2
	ceftriaxone	≤0.25	8	≤0.25–>32	90.5
	cefepime	≤0.12	0.5	≤0.12–>16	95.2
	co-amoxiclav	>16	>16	4–>16	4.8
	piperacillin/tazobactam	2	64	0.5–128	76.0
	aztreonam	≤0.12	2	≤0.12–>16	90.5
	imipenem	0.5	1	0.25–1	100.0
	meropenem	≤0.06	≤0.06	≤0.06–0.12	100.0
	gentamicin	≤1	>8	≤1–>8	85.7
	ciprofloxacin	≤0.25	2	≤0.25–2	85.7
	levofloxacin	0.12	1	≤0.03–2	100.0
Shigella spp. (12)	BAL9141	0.03	0.03	≤0.015–0.03	(100.0)
	cefazolin	≤2	4	≤2–4	100.0
	cefoxitin	2	4	1–4	100.0
	ceftazidime	≤2	≤2	≤2	100.0

Table 4. (*Continued*)

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) 50%	90%	range	% Susceptible[a]
	ceftriaxone	≤0.25	≤0.25	≤0.25–0.5	100.0
	cefepime	≤0.12	≤0.12	≤0.12–0.25	100.0
	co-amoxiclav	8	16	≤2–16	83.3
	piperacillin/tazobactam	2	4	0.25–16	91.7
	aztreonam	≤0.12	≤0.12	≤0.12–0.25	100.0
	imipenem	0.12	0.12	≤0.06–0.12	100.0
	meropenem	≤0.06	≤0.06	≤0.06–0.12	100.0
	gentamicin	≤1	2	≤1–2	100.0
	ciprofloxacin	≤0.015	≤0.015	≤0.015	100.0
	levofloxacin	≤0.03	≤0.03	≤0.03	100.0

[a]MIC interpretation criteria of the NCCLS.[27]
[b]A susceptibility breakpoint of ≤4 mg/L used for BAL9141 for comparison purposes only (% susceptible).
[c]Indole-positive *Proteae* include *P. vulgaris*, *Morganella* spp. and *Providencia* spp.

45.5% susceptibility at ≤4 mg/L and MIC_{50} values of 32 mg/L for *Acinetobacter* and nil susceptibility for *Burkholderia cepacia* (MIC_{50} 32 mg/L) and *Stenotrophomonas maltophilia* (MIC_{50} > 32 mg/L). In contrast, BAL9141 potency versus *P. aeruginosa* was equal (MIC_{90} 8 mg/L) to that of cefepime and ceftazidime (Table 6). The lower utilized breakpoint MIC for BAL9141 (≤4 mg/L) in these comparisons reduced its perceived anti-*Pseudomonas* spectrum. In contrast, the carbapenems demonstrated good activity against *P. aeruginosa* (90.5%), as did piperacillin/tazobactam (87%), amikacin, gentamicin and tobramycin (95.2–100.0%).

Most agents tested against *Acinetobacter* spp. demonstrated low susceptibility rates (Table 6), with the exception of tobramycin (78.9%) and the carbapenems (68.4%). *B. cepacia* demonstrated high rates of resistance to most agents tested, with the exception of the carbapenems (83.3–100.0%), levofloxacin (83.3%) and trimethoprim/sulfamethoxazole (100%). Of the agents tested against *S. maltophilia*, only piperacillin/tazobactam (82.4%), levofloxacin (82.4%) and trimethoprim/sulfamethoxazole (88.2%) showed an acceptable level of *in vitro* efficacy.

Activity of BAL9141 against Neisseria and anaerobes

Table 7 summarizes the BAL9141 activity compared with a limited number of antimicrobials tested by agar dilution methods against *N. meningitidis*, *N. gonorrhoeae* and 54 strains of anaerobic bacteria. BAL9141 proved to be two- to four-fold more active than cefotaxime against the pathogenic *Neisseria*. The highest MIC of BAL9141 was for the gonococcal strains at 0.06 mg/L (susceptible by criteria applied to either cefotaxime or ceftriaxone).

The *Clostridium* spp. strains were generally BAL9141-susceptible with an MIC_{90} of ≤0.25 mg/L, rates comparable to cefotaxime, but inferior to the comparison carbapenem. In contrast, *B. fragilis* group anaerobes were less susceptible to inhibition by BAL9141 (MIC_{90} > 64 mg/L). However, BAL9141 seems to be slightly more active than cefotaxime versus these *B. fragilis* group isolates (Table 7).

Discussion

Since the first reports of methicillin resistance in *S. aureus* nearly four decades ago,[31] pharmaceutical scientists have sought β-lactam molecules active against these strains. Numerous candidate agents have been studied, many with high affinities for the *mecA* gene product (PBP2a) responsible for the resistance.[32] Structurally diverse β-lactams have been developed to address this need, including carbapenems[14,15,32–34] and numerous cephalosporin derivat- ives,[14–17,21,22] including BAL9141. In this report, we confirm the potency of BAL9141 against MR staphylococci, including multidrug-resistant strains and those organisms with elevated glycopeptide MICs.[3,4] Specifically, BAL9141 was eight-fold more active than ceftriaxone against OS staphylococci and no MRSA had a BAL9141 MIC of >2 mg/L, validating the report by Hebeisen *et al.*[21] and showing activity similar to that of RWJ-54428.[16]

Although highly promising against MR staphylococci, the BAL9141 activity versus penicillin-resistant *S. pneumoniae* (MIC_{90} 0.25 mg/L), vancomycin-susceptible and -resistant *E. faecalis* (MIC_{90} 4 mg/L), penicillin-resistant viridans group streptococci (MIC_{90} 1 mg/L), *H. influenzae* (MIC_{90} 0.06–0.5 mg/L) and *M. catarrhalis* (MIC_{90} 0.5 mg/L) was considered excellent. Against the Enterobacteriaceae, the anti-bacterial spectrum of BAL9141 most closely resembled

Table 5. Antimicrobial activity of BAL9141 (formerly Ro63-9141) and 13 other comparison drugs tested against *E. coli* and *K. pneumoniae* isolates having an ESBL phenotype[a]

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) 50%	90%	range	% Susceptible[b]
E. coli (23)[c]	BAL9141	32	>32	0.03–>32	(26.1)[d]
	cefoxitin	4	>32	2–>32	69.6
	ceftazidime	16	>32	0.25–>16	43.5
	ceftriaxone	32	>32	≤0.25–>32	26.1
	cefepime	4	>16	≤0.12–>16	73.9
	piperacillin/tazobactam	4	128	0.5–>128	78.3
	aztreonam	>16	>16	≤0.12–>16	26.1
	imipenem	0.12	0.12	≤0.06–0.5	100.0
	meropenem	≤0.06	≤0.06	≤0.06–1	100.0
	tobramycin	>16	>16	0.5–>16	34.8
	ciprofloxacin	≤0.25	>2	≤0.25–>2	52.2
	tetracycline	>8	>8	≤4–>8	8.7
	trimethoprim/sulfamethoxazole	>1	>1	≤0.5–>1	17.4
K. pneumoniae (25)	BAL9141	>32	>32	≤0.015–>32	(12.0)
	cefazolin	>16	>16	>16	0.0
	cefoxitin	4	32	2–>32	80.0
	ceftazidime	>16	>16	1–>16	24.0
	cefepime	4	>16	0.5–>16	72.0
	piperacillin/tazobactam	128	>128	2–>128	44.0
	aztreonam	>16	>16	1–>16	16.0
	imipenem	0.12	0.5	0.12–1	100.0
	meropenem	≤0.06	0.25	≤0.06–1	100.0
	tobramycin	>16	>16	0.25–>16	24.0
	ciprofloxacin	≤0.25	>2	≤0.25–>2	88.0
	tetracycline	≤4	>8	≤4–>8	68.0
	trimethoprim/sulfamethoxazole	1	>2	≤0.5–>2	48.0

[a]ESBL phenotype defined by the NCCLS[27] as a MIC of ≥2 mg/L for aztreonam or ceftazidime or ceftriaxone and an inhibitable enzyme by clavulanic acid.
[b]Susceptible interpretative criteria as published by the NCCLS.[27]
[c]Includes three strains that had cefoxitin MICs > 32 mg/L, consistent with an ampC enzyme.
[d]Percentage in parentheses indicates the proportion of strains at ≤4 mg/L of BAL9141 (for comparison purposes only).

that of cefepime, although some strains of *Enterobacter* spp., indole-positive *Proteae* (*P. vulgaris*) and *Serratia* appear to be more resistant towards BAL9141. ESBL-producing isolates of *E. coli* and *K. pneumoniae* were not inhibited by BAL9141 ($MIC_{50} \geq 32$ mg/L), suggesting rapid hydrolysis by these enzymes (Table 5). All tested non-fermentative Gram-negative bacilli (except *P. aeruginosa*) were highly resistant to BAL9141. Cefepime, ceftazidime and BAL9141 had essentially identical activities against *P. aeruginosa* isolates, and the breakpoint selected by pharmacokinetic/pharmacodyamic analyses (≤4 or ≤8 mg/L) will have a great influence on the role of BAL9141 for therapy of infections caused by this species. At a susceptible breakpoint equal to that of other parenteral cephalosporins, >90% of *P. aeruginosa* would be judged as BAL9141 treatable, but only 69.6% of strains at ≤4 mg/L. Like many third- and fourth-generation cephalosporins, BAL9141 was very active against pathogenic *Neisseria* (MIC_{90} 0.004–0.06 mg/L) and Gram-positive anaerobic bacteria ($MIC_{90} \leq 0.25$ mg/L).

Since all currently marketed β-lactams are considered clinically inactive against MR staphylococci,[25,27] the probability of adverse selection of resistant strains in the hospital environment using cephalosporins remains high. The concurrent co-resistance of these MR staphylococci to the most popularly used parenteral fluoroquinolones (ciprofloxacin, levofloxacin) contributes to an even greater risk of escalating rates of MRSA isolation. The clinical availability of a cephalosporin candidate (BAL9141) with MRSA activity presents the option of a therapeutic broad-spectrum cephalosporin coupled with environmental (patient or hospital) suppression of MR staphylococci. Indeed, *in vivo* animal model results[21] suggest that BAL9141 could be utilized as directed therapy of MRSA infections, including those possessing reduced susceptibility to vancomycin.

Table 6. Antimicrobial activity of BAL9141 (formerly Ro63-9141) and 13 other comparison drugs tested against non-fermentative Gram-negative bacilli

Organism (no. tested)	Antimicrobial agent	MIC (mg/L)			% Susceptible[a]
		50%	90%	range	
Acinetobacter spp. (22)	BAL9141	32	>32	≤0.015–>32	(45.5)[b]
	ceftazidime	>16	>16	≤2–>16	36.8
	ceftriaxone	>32	>32	≤0.25–>32	26.3
	cefepime	>16	>16	≤0.12–>16	36.8
	piperacillin/tazobactam	32	>128	≤0.06–>128	50.0
	aztreonam	>16	>16	0.5–>16	10.5
	imipenem	0.5	>8	≤0.06–>8	68.4
	meropenem	2	>8	≤0.06–>8	68.4
	amikacin	2	>32	0.5–>32	63.2
	gentamicin	2	>8	≤1–>8	57.9
	tobramycin	1	>16	0.25–>16	78.9
	ciprofloxacin	>2	>2	0.03–>2	31.6
	levofloxacin	>4	>4	≤0.03–>4	36.8
	trimethoprim/sulfamethoxazole	>2	>2	≤0.5–>2	42.1
B. cepacia (8)	BAL9141	32	–	16–>32	(0.0)
	ceftazidime	2	–	≤2–>16	66.7
	ceftriaxone	8	–	8–>16	50.0
	cefepime	4	–	4–>16	66.7
	piperacillin/tazobactam	128	–	16–>128	37.5
	aztreonam	8	–	8–>16	50.0
	imipenem	4	–	2–4	100.0
	meropenem	2	–	1–8	83.3
	amikacin	>32	–	8–>32	16.7
	gentamicin	>8	–	8–>8	0.0
	tobramycin	>16	–	4–>16	16.7
	ciprofloxacin	1	–	0.5–2	66.7
	levofloxacin	2	–	0.5–4	83.3
	trimethoprim/sulfamethoxazole	≤0.5	–	≤0.5	100.0
P. aeruginosa (23)	BAL9141	2	8	0.12–16	(69.6)
	ceftazidime	≤2	8	≤2–>16	90.5
	ceftriaxone	32	>32	≤0.25–>32	33.3
	cefepime	2	8	≤0.12–16	90.5
	piperacillin/tazobactam	4	>128	0.25–>128	87.0
	aztreonam	4	>16	≤0.12–>16	71.4
	imipenem	0.5	2	0.12–8	90.5
	meropenem	0.5	4	≤0.06–>8	90.5
	amikacin	2	8	≤0.25–8	100.0
	gentamicin	2	4	≤1–>8	95.2
	tobramycin	0.5	1	≤0.12–>16	95.2
	ciprofloxacin	0.25	>2	≤0.015–>2	71.4
	levofloxacin	1	>4	0.12–>4	66.7
	trimethoprim/sulfamethoxazole	>2	>2	≤0.5–>2	14.3
S. maltophilia (17)	BAL9141	>32	>32	>32	(0.0)
	ceftazidime	>16	>16	1–>16	35.3
	ceftriaxone	>32	>32	16–>32	0.0
	cefepime	16	>16	4–>16	23.4
	piperacillin/tazobactam	64	>128	16–>128	82.4
	aztreonam	>16	>16	8–>16	5.9
	imipenem	>8	>8	>8	0.0
	meropenem	>8	>8	2–>8	6.9
	amikacin	>32	>32	8–>32	23.5
	gentamicin	>8	>8	≤1–>8	23.5

Table 6. (*Continued*)

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) 50%	90%	range	% Susceptible[a]
	tobramycin	16	>16	2–>16	23.5
	ciprofloxacin	2	>2	0.5–>2	29.4
	levofloxacin	1	4	0.25–>4	82.4
	trimethoprim/sulfamethoxazole	≤0.5	1	≤0.5–>1	88.2

[a]NCCLS breakpoint criteria were used as applied to *P. aeruginosa*.[27]
[b]For BAL9141, a susceptible breakpoint of ≤4 mg/L was utilized for comparison purposes only (% found in parentheses).

Table 7. Antimicrobial activity of BAL9141 compared with cefotaxime and meropenem when testing pathogenic *Neisseria* and strict anaerobic bacteria

Organism (no. tested)	Antimicrobial agent	MIC (mg/L) 50%	90%	range	% inhibited at MIC (mg/L) ≤1	≤2	≤4	≤8
N. meningitidis (24)	BAL9141	≤0.002	0.004	≤0.002–0.008	100	100	100	100
	cefotaxime	0.008	0.016	≤0.004–0.016	100	100	100	100
N. gonorrhoeae (32)[a]	BAL9141	0.03	0.06	0.008–0.06	100	100	100	100
	cefotaxime	0.03	0.12	≤0.004–0.25	100	100	100	100
B. fragilis group (44)[b]	BAL9141	8	>64	≤0.25–>64	16	23	36	61
	cefotaxime	32	>128	≤0.25–>128	11	14	18	20
	meropenem	0.12	0.25	≤0.03–8	95	98	98	100
Clostridium spp. (10)[c]	BAL9141	≤0.25	≤0.25	≤0.25–64	90	90	90	90
	cefotaxime	2	4	≤0.25–32	40	70	90	90
	meropenem	0.06	0.06	≤0.03–0.12	100	100	100	100

[a]Includes strains resistant to ciprofloxacin (seven) and penicillin by target mutation or β-lactamase production (16 strains).
[b]Includes *B. distasonis* (one strain), *B. fragilis* (28 strains), *B. splanchnicus* (one strain), *B. thetaiotaomicron* (six strains), *B. vulgatus* (two strains) and *B. fragilis* group (six strains).
[c]Includes *C. butyricum* (one strain), *C. difficile* (one strain), *C. lipolytica* (one strain) and *C. perfringens* (seven strains).

Results of initial clinical trials as well as human pharmacokinetic results leading to the establishment of a reliable breakpoint for BAL9141 susceptibility are eagerly awaited. Early trials appear warranted to follow the effects of therapy on patient colonization by resistant Gram-positive pathogens and changes in normal flora.

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