

# Antimicrobial Susceptibilities of Clinical Isolates of HACEK Organisms

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**The “HACEK” organisms are a group of fastidious Gram-negative bacteria that cause a variety of infections, including infective endocarditis. Antimicrobial susceptibility testing is not universally available, and therapy for these infections is often empirical. We report the antimicrobial susceptibilities of 70 clinical HACEK isolates to 18 antimicrobials. All isolates were susceptible to ceftriaxone and levofloxacin, indicating that these agents remain appropriate empirical choices for the treatment of infections with this group of organisms.**

The “HACEK” group of fastidious Gram-negative organisms includes *Haemophilus* species (other than *Haemophilus influenzae*), *Aggregatibacter actinomycetemcomitans* (formerly *Actinobacillus actinomycetemcomitans*), *Aggregatibacter aphrophilus* (formerly *Haemophilus aphrophilus*), *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*. These organisms are normal flora of the human oral cavity, but are capable of causing disease, most notably infective endocarditis (IE), but also periodontal infections, abscesses, and nonendocarditis bacteremia secondary to focal infections.

Since these organisms are fastidious, antimicrobial susceptibility testing is often difficult and impractical, particularly for primary/non-reference laboratories. Therefore, therapy is often chosen empirically based on published reports and guidelines.

North American and European IE guidelines recognize that the likelihood of ampicillin resistance in HACEK organisms precludes empirical therapy with ampicillin (1–3). The recommended treatment of IE due to these organisms is therefore a broad-spectrum cephalosporin or a fluoroquinolone. This is based on limited data due to the clinical rarity of infections by these organisms. To our knowledge, only one study has described the antimicrobial susceptibilities of multiple genera of HACEK organisms, which included 42 clinical and American Type Culture Collection (ATCC) strains (4).

The Public Health Ontario Laboratory—Toronto (PHOL) is a provincial reference laboratory for antimicrobial susceptibility testing in the province of Ontario, Canada, with a catchment area of 13.5 million people. HACEK organisms are sent to this laboratory from primary, tertiary, and quaternary care centers for identification and/or susceptibility testing. From January 2010 to July 2012, PHOL received 241 HACEK isolates for identification and/or susceptibility testing. Of those, 49 isolates were submitted for identification only and 18 isolates were recovered from autopsy specimens, which do not routinely undergo susceptibility testing. The remaining 174 isolates were submitted for identification and susceptibility testing. Isolates were identified using either traditional biochemical tests or the 16S rRNA molecular assay (5). Antimicrobial susceptibility testing was performed using broth microdilution with cation-adjusted Mueller-Hinton broth (CAMHB) containing 5% (vol/vol) lysed horse blood (LHB) per Clinical and Laboratory Standards Institute (CLSI) M45-A2

guidelines (6). After 48 h of incubation, results were interpreted using breakpoints published by CLSI (6).

Of 174 isolates, 104 (59.8%) isolates failed to grow adequately in the control well and therefore were not able to provide valid susceptibility results. After two attempts, results were reported as “unable to perform susceptibility testing.” Of these isolates, 10 out of 12 (83.3%) *A. actinomycetemcomitans*, 17 out of 28 (60.7%) *A. aphrophilus*, 3 out of 5 (60%) *C. hominis*, 18 out of 55 (32.7%) *Haemophilus parainfluenzae*, 55 out of 72 (76.4%) *E. corrodens*, and 1 out of 2 (50%) *K. kingae* isolates failed susceptibility testing.

Among the isolates with successful susceptibility testing, the most common isolates were *H. parainfluenzae* (37 isolates), followed by *E. corrodens* (17 isolates) and *A. aphrophilus* (11 isolates). Two isolates each of *C. hominis* and *A. actinomycetemcomitans* and one isolate of *K. kingae* were tested. Blood isolates accounted for 31.4% of samples that underwent successful susceptibility testing, abscess fluid accounted for 28.6%, and other fluid (including cerebrospinal fluid, synovial fluid, and peritoneal fluid as well as unspecified fluid samples) accounted for 25.7%, while the remaining samples were tissue or unspecified samples.

The MICs of 18 antimicrobial agents for 70 isolates are summarized in Table 1. No isolates were resistant to amoxicillin-clavulanic acid, ceftriaxone, meropenem, levofloxacin, or chloramphenicol based on CLSI breakpoints (Table 1). Clarithromycin and penicillin were the least active agents, with 44.3% and 22.9% of isolates being nonsusceptible, respectively. Resistance to imipenem, sulfamethoxazole-trimethoprim, ampicillin, and tetracycline was also observed. Notably, two isolates—one pleural fluid isolate of *H. parainfluenzae* and one blood isolate of *A. actinomycetemcomitans*—were resistant to ampicillin-sulbactam. Both isolates were positive for  $\beta$ -lactamase activity as determined by the cefinase disk method.

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TABLE 1 MICs of antimicrobial agents against clinical HACEK isolates in this study<sup>a</sup>

Drug(s)	All isolates (n = 70)		<i>A. actinomycetemcomitans</i> (n = 2)		<i>A. aphrophilus</i> (n = 11)		<i>H. parainfluenzae</i> (n = 37)		<i>C. hominis</i> (n = 2)		<i>E. corrodens</i> (n = 17)		<i>K. kingae</i> (n = 1)	
	MIC range, $\mu\text{g/ml}$ (% S)	MIC <sub>50/90</sub> <sup>b</sup> , $\mu\text{g/ml}$	MIC range, $\mu\text{g/ml}$ (% S)	MIC <sub>50/90</sub> <sup>b</sup> , $\mu\text{g/ml}$	MIC range, $\mu\text{g/ml}$ (% S)	MIC <sub>50/90</sub> <sup>b</sup> , $\mu\text{g/ml}$	MIC range, $\mu\text{g/ml}$ (% S)	MIC <sub>50/90</sub> <sup>b</sup> , $\mu\text{g/ml}$	MIC range, $\mu\text{g/ml}$ (% S)	MIC <sub>50/90</sub> <sup>b</sup> , $\mu\text{g/ml}$	MIC range, $\mu\text{g/ml}$ (% S)	MIC <sub>50/90</sub> <sup>b</sup> , $\mu\text{g/ml}$	MIC range, $\mu\text{g/ml}$ (% S)	MIC <sub>50/90</sub> <sup>b</sup> , $\mu\text{g/ml}$
<b>Penicillins</b>														
<b>Amoxicillin-clavulanic acid</b>														
Ampicillin-sulbactam	$\leq 1-2$ (97.1)	1/1	$\leq 1-2$ (50)	ND <sup>d</sup>	$\leq 2$ (100)	2	$\leq 2$ (100)	2	$\leq 2$ (100)	ND	ND	2	S	$\leq 2$
Ampicillin	$\leq 0.12-2$ (95.8)	0.25/1	0.5-4 (50)	ND	$\leq 1$ (100)	1/1	$\leq 1-2$ (97.3)	1/2	$\leq 1$ (100)	ND	ND	1/1	S	$\leq 1$
Penicillin	$\leq 0.015-2$ (77.1)	0.5/1	1-2 (50)	ND	$\leq 0.12-1$ (100)	0.5/0.5	$\leq 0.12-4$ (97.3)	0.25/1	$\leq 0.12-0.25$ (100)	ND	ND	0.25/1	S	$\leq 0.12$
					$\leq 0.015-2$ (81.8)	0.5/1	$\leq 0.015-2$ (67.6)	1/1	0.06-0.12 (100)	ND	ND	0.5/1	S	0.03
<b>Cephalosporins</b>														
<b>Ceftriaxone</b>														
Cefixime	$\leq 0.03-0.5$ (100)	0.03/0.12	$\leq 0.03-0.25$ (100)	ND	$\leq 0.03$ (100)	0.03/0.03	$\leq 0.03-0.5$ (100)	0.03/0.03	0.06-0.25 (100)	ND	ND	0.03/0.12	S	$\leq 0.03$
Cefepime	$\leq 0.12-2$	0.12/0.25	$\leq 0.12-0.25$	ND	$\leq 0.12-0.25$	0.12/0.12	$\leq 0.12-0.5$	0.12/0.12	$\leq 0.12-1$	ND	ND	0.12/1	S	$\leq 0.12$
Cefuroxime	$\leq 0.12-2$	0.12/0.5	0.25-2	ND	$\leq 0.12-0.25$	0.12/0.12	$\leq 0.12-2$	0.12/0.5	$\leq 0.12-0.5$	ND	ND	0.12/0.5	S	$\leq 0.12$
Ceftazidime	$\leq 0.5-8$	0.5/2	$\leq 0.5-2$	ND	$\leq 0.5$	0.5/0.5	$\leq 0.5-1$	0.5/1	$\leq 0.5-8$	ND	ND	2/8	S	$\leq 0.5$
Cefticlor <sup>b</sup>	$\leq 4-16$	4/4 <sup>b</sup>	$\leq 4$	ND	$\leq 4$	4/4	$\leq 4-16$	4/4	$\leq 4$	ND	ND	4/8 <sup>b</sup>	S	$\leq 4$
<b>Carbapenems</b>														
<b>Meropenem</b>														
Imipenem	$\leq 0.06-0.25$ (100)	0.06/0.06	$\leq 0.06-0.25$ (100)	ND	$\leq 0.06$ (100)	0.06/0.06	$\leq 0.06$ (100)	0.06/0.06	$\leq 0.06$ (100)	ND	ND	0.06/0.06	S	$\leq 0.06$
	$\leq 0.5-1$ (ND) <sup>c</sup>	0.5/0.5	$\leq 0.5-1$ (100)	ND	$\leq 0.5$ (100)	0.5/0.5	$\leq 0.5-1$ (97.3)	0.5/0.5	$\leq 0.5$ (100)	ND	ND	0.5/0.5	S	$\leq 0.5$
<b>Fluoroquinolones</b>														
<b>Levofloxacin</b>														
Sparfloxacin	$\leq 0.03-0.25$ (100)	0.03/0.12	$\leq 0.03$ (100)	ND	$\leq 0.03-0.06$ (100)	0.03/0.06	$\leq 0.03-0.25$ (100)	0.03/0.12	$\leq 0.03-0.25$ (100)	ND	ND	0.03/0.12	S	$\leq 0.03$
	$\leq 0.03-1$	0.03/0.12	$\leq 0.03$	ND	$\leq 0.03-0.25$	0.03/0.06	$\leq 0.03-1$	0.03/0.12	$\leq 0.03$	ND	ND	0.03/0.06	S	$\leq 0.03$
<b>Other agents</b>														
<b>Chloramphenicol</b>														
Tetracycline	$\leq 0.5-2$ (100)	1/2	$\leq 0.5-1$ (100)	ND	$\leq 0.5-2$ (100)	0.5/1	$\leq 0.5-2$ (100)	0.5/2	$\leq 0.5-1$ (100)	ND	ND	1/2	S	$\leq 0.5$
Sulfamethoxazole-trimethoprim <sup>b</sup>	$\leq 0.25-2$ (98.6)	1/1	0.5-1 (100)	ND	$\leq 0.25-2$ (100)	0.25/2	$\leq 0.25-4$ (97.3)	0.25/2	$\leq 0.25-5$	ND	ND	0.5/1	S	$\leq 0.25$
Clarithromycin	$\leq 0.06-2$ (93.2) <sup>b</sup>	0.06/0.25 <sup>b</sup>	$\leq 0.06$ (100)	ND	$\leq 0.06-0.25$ (100) <sup>b</sup>	0.06/0.25 <sup>b</sup>	$\leq 0.06-2$ (86.2) <sup>b</sup>	0.06/1 <sup>b</sup>	$\leq 0.06-0.25$ (100)	ND	ND	0.06/0.5	S	$\leq 0.06$
Erythromycin	$\leq 0.12-16$ (55.7)	4/16	8-16 (50)	ND	$\leq 0.12-16$ (54.5)	0.12/16	$\leq 0.12-16$ (32.4)	16/16	$\leq 0.12-8$ (100)	ND	ND	2/8	S	0.25
	$\leq 0.25-2$ (50)	$>0.5$	$>0.5$	ND	$>0.5$	$>0.5$	$\leq 0.25-2$ (50)	$>0.5$	$\leq 0.25-2$ (50)	ND	ND	$>0.5$	S	0.5

<sup>a</sup> Susceptibility testing was performed using the methodology recommended in the CLSI M45-A2 guidelines (6). Results for drugs to which all bacteria were susceptible (S) have been highlighted in boldface. A single number in the

<sup>b</sup> MIC<sub>50/90</sub><sup>b</sup> column represents the MIC<sub>90</sub>.

<sup>c</sup> Sensitive MICs were indeterminate; therefore, some data were excluded.

<sup>d</sup> ND, not determined.

<sup>e</sup> -, CLSI breakpoints are not available.

The penicillin with the greatest *in vitro* activity was amoxicillin-clavulanic acid. The relative potencies of the remaining penicillins by MIC<sub>90</sub> were ampicillin-sulbactam > ampicillin > penicillin. Ceftriaxone was active against all isolates and was also the most potent cephalosporin (MIC<sub>90</sub>, 0.12 µg/ml). The relative potencies of the remaining cephalosporins tested were cefixime > cefepime > cefuroxime > cefaclor. Meropenem was more active and more potent than imipenem. Levofloxacin and chloramphenicol were universally active.

Resistance was most frequently observed in *Aggregatibacter* and *Haemophilus* species. Six of 13 *Aggregatibacter* species were resistant to at least one agent, and 3 of 13 were resistant to at least two agents. Twenty-five of 37 *Haemophilus* isolates were intermediate or resistant to at least one agent, and 16 were intermediate or resistant to more than one agent. Of the remaining 20 isolates, one *Eikenella* isolate was resistant to ampicillin and penicillin. Notably, this isolate did not produce a β-lactamase. The two *Cardiobacterium* isolates and a single *Kingella* isolate were susceptible to all tested antimicrobials.

From this data set, the following observations can be made. (i) Using recommended methods, antimicrobial susceptibility testing for a significant proportion of HACEK isolates may not be possible. (ii) Broad-spectrum cephalosporins and fluoroquinolones are consistently active against HACEK organisms *in vitro* (based on breakpoints from published guidelines [6]). (iii) The combination ampicillin-sulbactam (recommended in the IE guidelines of the Infectious Disease Society of America/American Heart Association) was not universally active against these isolates and therefore may not be appropriate empirical therapy in all settings (1). (iv) A significant number of *Aggregatibacter* and *Haemophilus* species are resistant to multiple antimicrobials. (v) Penicillin/ampicillin resistance was present in one isolate of *Eikenella corrodens* in the absence of β-lactamase production.

Using contemporary isolates, our data corroborate those from a smaller published series of clinical and ATCC HACEK strains in which MICs for 29 antimicrobials were generally low (4). Our data are most robust for isolates of *Aggregatibacter*, *Haemophilus*, and *Eikenella*, since only a limited number of *Cardiobacterium* and *Kingella* isolates were tested. Reports of series of clinical isolates of *Cardiobacterium* and *Kingella* have shown low MICs for β-lactams and cephalosporins and universal susceptibility to fluoroquinolones, suggesting that, consistent with our findings, resistance among these organisms to broad-spectrum cephalosporins or fluoroquinolones is unlikely (7, 8).

Since the fastidiousness of HACEK organisms makes resistance testing impractical for many laboratories, and isolates commonly

fail susceptibility testing, published reports and guidelines frequently provide the only guidance for antimicrobial selection (1–3). The high failure rate for susceptibility results among these organisms also indicates that current recommended susceptibility methods may not be optimal. Our findings confirm that the use of broad-spectrum cephalosporins and fluoroquinolones remains appropriate for this group of organisms.

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