The Role of Whole Genome Sequencing on Post-Marketing Surveillance Programs: Results of the INFORM Surveillance Program for Ceftazidime-Avibactam in the United States

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INTRODUCTION

- Post-marketing *in vitro* surveillance programs are performed to comply with the requirements of regulatory agencies responsible for approving new antimicrobial agents, such as the United States Food and Drug Administration (US FDA) and the European Medicine Agency (EMA)
- Comprehensive post-marketing *in vitro* surveillance programs are essential for detecting the emergence of resistance and changes in resistance patterns that may occur after the clinical introduction and use of new antimicrobial agents
- Furthermore, data generated by these programs can assist clinicians beyond the scope of local antibiograms, which may not be available for any given new drug, by providing useful information on the potency and spectrum of new antimicrobial agents in which clinicians have limited or no experience
- The power of genome sequencing, bioinformatic tools, and curated databases for *in silico* analysis can provide organism identification, a better understanding of antimicrobial resistance, distribution of resistance and virulence genes, and epidemiology of bacterial populations
- A well-designed surveillance system combined with molecular characterization of isolates provides pivotal information on the prevalence of clinically significant resistance genotypes and subsequent changes over time
  - Monitoring for resistance determinants can also help detect new, emerging resistance mechanisms and evaluate the dissemination of resistance determinants and clones
- We evaluated the results obtained by whole genome sequencing (WGS) and *in silico* screening of β-lactamase-encoding genes from 2,600 *Enterobacterales* isolates tested as part of the International Network for Optimal Resistance Monitoring (INFORM) surveillance program for ceftazidime-avibactam in the United States

MATERIALS AND METHODS

Bacterial isolates

- A total of 19,535 *Enterobacterales* isolates causing infections in hospitalized patients in the United States were collected as part of the INFORM Program in 2016-2017 (Table 1)
  - These isolates were consecutively collected (1 per patient) from 85 sites located in 37 states in 9 US census divisions and were submitted to JMI Laboratories (North Liberty, Iowa, USA)
- Isolates were initially identified by the participating laboratory, and identifications were confirmed at JMI Laboratories using matrix assisted laser desorption ionization time of flight technology mass spectrometry (Bruker Daltonics, Bremen, Germany) and/or genome sequencing

Antimicrobial susceptibility testing

- Isolates were tested for susceptibility by broth microdilution using frozen-form broth microdilution panels containing cation-adjusted Mueller-Hinton broth and manufactured by JMI Laboratories according to CLSI M07 (2018)

Screen for β-lactamase genes by WGS and bioinformatic tools

- Selection criteria:
  - *Escherichia coli* and *Klebsiella* spp. isolates displaying elevated MIC results (≥2 mg/L) for ceftriaxone, aztreonam, ceftazidime, or imipenem/meropenem (MIC, ≥2 mg/L)
  - *Enterobacter cloacae* species complex (herein *E. cloacae*), *E. aerogenes* (2016 only), and *Citrobacter* spp. isolates displaying MIC values ≥16 mg/L for ceftazidime and/or ≥2 mg/L for cefepime
  - *Enterobacterales* isolates displaying imipenem (imipenem was **not** applied to *Proteus mirabilis* or to indole-positive Proteeae), meropenem, or doripenem MIC results ≥2 mg/L
  - *Enterobacterales* isolates displaying ceftazidime-avibactam MIC values >4 mg/L
- A total of 2,600 isolates met the MIC screening criteria and were submitted to WGS and analysis (Table 1)
- Total genomic DNA was extracted using the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, Ohio, USA)
- DNA samples were quantified using the Qubit™ High Sensitivity DS-DNA assay (Invitrogen, ThermoFisher Inc.) and normalized to 0.2 ng/μL
  - A total of 1 ng high-quality genomic DNA was used as input material for library construction
- DNA libraries were prepared using the Nextera XT™ library construction protocol (Illumina, San Diego, California, USA) following the manufacturer's instructions and sequenced on a MiSeq Sequencer (Illumina) at JMI Laboratories
- Each raw sequencing data set was quality assured, error corrected, and assembled *de novo* using the SPAdes genome assembler (v 3.9.0)
- Assembled genomes were subjected to a proprietary software (JMI Laboratories) to screen for known β-lactamase-encoding genes

RESULTS

- Among 10,546 and 9,079 *Enterobacterales* isolates collected in 2016 and 2017, respectively, 1,362 (13.0%) and 1,238 (13.6%) were submitted to WGS (Table 1)
- An extended-spectrum β-lactamase (ESBL)-encoding gene was detected in 13.0% and 14.3% of *E. coli* and 10.2% and 11.5% of *K. pneumoniae* isolates in 2016 and 2017, respectively (Table 2)
- The most common ESBLs were CTX-M type, which were observed in 97.9% and 76.3% of ESBL-producing *E. coli* and *K. pneumoniae*, respectively (Table 2)
- Overall, a CTX-M gene was detected in 6.6%/7.3% of *Enterobacterales* isolates in 2016/2017 (Table 3), including 12.7%/14.0% of *E. coli* and 7.8%/8.7% of *K. pneumoniae* (Tables 2 - 4)
- OXA-type enzymes (mainly OXA-1/30-like) were the second most common β-lactamase group and were detected in 3.2%/3.3% of *Enterobacterales* in 2016/2017, including 5.3%/5.1% of *E. coli* and 4.9%/5.5% of *K. pneumoniae* isolates (Table 4)
- A carbapenemase gene was detected in 1.1% and 0.9% of *Enterobacterales* in 2016 and 2017, respectively, and *K. pneumoniae* carbapenemase (KPC)-producing isolates represented 95.0% of the carbapenemase producers (Tables 2 and 3)
- The prevalence of KPC-producing *K. pneumoniae* isolates decreased from 3.4% in 2016 to 2.2% in 2017 (Tables 2 - 4)
- A metallo-β-lactamase (MBL) was observed in only 2 isolates (0.01%; NDM-1 and IMP-27), both in 2016 (Tables 3 and 4)
- Ceftazidime avibactam was very active against ESBL-producing strains ($MIC_{50/90}$, 0.12-0.25/0.5 mg/L), including CTX-M producers ($MIC_{50/90}$, 0.12/0.5 mg/L), OXA-ESBL producers ($MIC_{50/90}$, 0.12-0.25/0.5 mg/L), and SHV-ESBL producers ($MIC_{50/90}$, 0.25/1 mg/L; Table 5)
- Ceftazidime avibactam also exhibited potent *in vitro* activity against transferable AmpC producers ($MIC_{50/90}$, 0.25/0.5-1 mg/L) and serine carbapenemase producers ($MIC_{50/90}$, 0.5/2 mg/L; Table 5 and Figure 1)
- Only 5 of 19,535 (0.03%) *Enterobacterales* isolates evaluated by the INFORM Program in 2016-2017 were resistant (MIC, >8 mg/L) to ceftazidime-avibactam (2 *E. cloacae* and 2 *P. stuartii* from 2016 and 1 *Serratia marcescens* from 2017; Table 6)

CONCLUSIONS

- A slight increase in ESBL-producing *E. coli* and *K. pneumoniae* and a decrease in KPC-producing *K. pneumoniae* isolates were observed in 2017 compared to 2016
- MBL-producing *Enterobacterales* isolates remain very rare in the United States
- Ceftazidime-avibactam retained potent *in vitro* activity against β-lactamase-producing strains isolated in US medical centers in 2016-2017
- WGS combined with bioinformatics for data analysis represent valuable tools for monitoring resistance mechanism trends in post-marketing surveillance

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Figure 1 Ceftazidime-avibactam MIC distribution for serine carbapenemase and ESBL-producing strains (INFORM Program, 2016-2017)

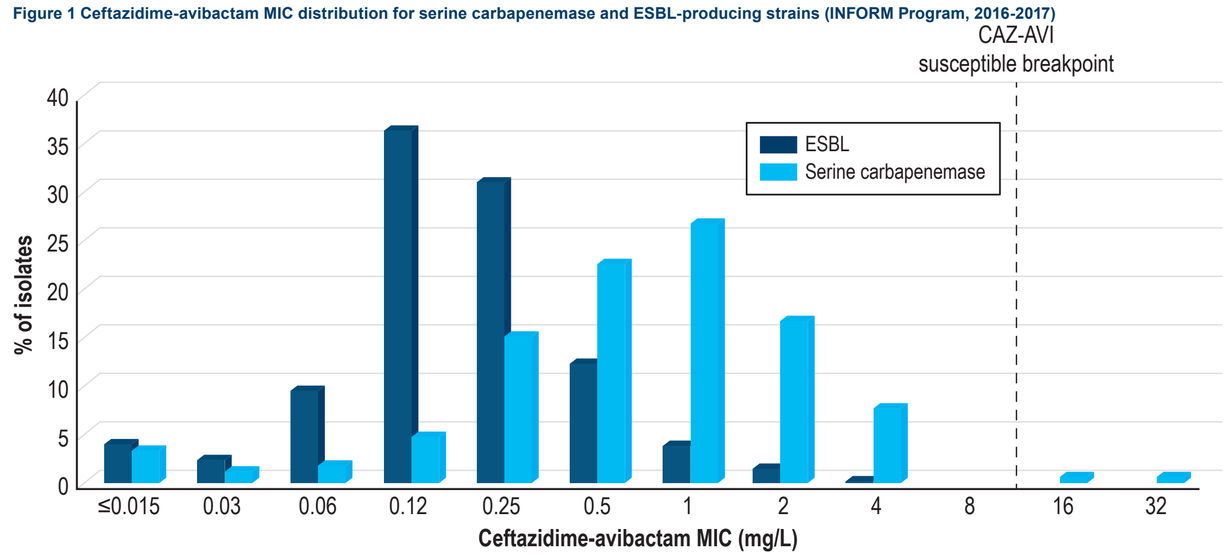

Table 1 Counts of isolates included in the INFORM Program and characterized by whole genome sequencing stratified by year and species

Organism	2016			2017		
	Isolates included in the program	Isolates screened	Isolates with positive results (% of screened)	Isolates included in the program	Isolates screened	Isolates with positive results (% of screened)
Enterobacterales	10,456	1,362	980 (72.0%)	9,079	1,238	993 (80.9%)
E. coli	3,751	558	547 (98.0%)	3,422	586	567 (96.6%)
K. pneumoniae	2,201	293	291 (99.3 %)	1,834	264	261 (98.9%)
K. oxytoca	480	59	25 (42.4%)	382	40	13 (32.5%)
E. cloacae	1,021	264	84 (31.8%)	911	235	84 (35.7%)
E. aerogenes	427	88	4 (4.5%)	367	25	25 (100.0%)
Citrobacter spp.	551	71	18 (25.3%)	499	69	23 (33.3%)
Other species	2,025	29	11 (37.9%)	1,664	19	18 (94.7%)

Table 2 Occurrence of the main β-lactamase groups among *E. coli*, *K. pneumoniae*, and all *Enterobacterales* isolates combined

β-lactamase	2016 (no. of isolates / % of total)			2017 (no. of isolates / % of total)		
	E. coli	*K. pneumoniae*	All	*E. coli*	*K. pneumoniae*	All
ESBLs	488 (13.0%)	224 (10.2%)	759 (7.3%)	491 (14.3%)	211 (11.5%)	743 (8.2%)
CTX-M	478 (12.7%)	172 (7.8%)	685 (6.6%)	480 (14.0%)	160 (8.7%)	667 (7.3%)
Carbapenemase	4 (0.1%)	75 (3.4%)	114 (1.1%)	6 (0.2%)	42 (2.3%)	86 (0.9%)
KPC	4 (0.1%)	74 (3.4%)	109 (1.0%)	6 (0.2%)	41 (2.2%)	81 (0.9%)
Transferable AmpC	48 (1.3%)	15 (0.7%)	75 (0.7%)	73 (2.1%)	10 (0.5%)	92 (1.0%)

ESBL, extended-spectrum β-lactamase.

Table 3 Occurrence of β-lactamase stratified for the entire *Enterobacterales* collection and year

Organism/β-lactamase class/β-lactamase gene (total no. 2016/2017)[a]	2016			2017		
	No.[b]	% of total[c]	% of screened[d]	No.[b]	% of total[c]	% of screened[d]
***Enterobacterales* (10,456/9,079)[a]**						
ESBLs	**759**	**7.3%**	**55.7%**	**743**	**8.2%**	**60.0%**
CTX-M	685	6.6%	51.9%	667	7.3%	56.5%
CTX-M-1 group	527	5.0%	40.0%	506	5.6%	42.8%
CTX-M-9 group	159	1.5%	12.1%	165	1.8%	14.0%
OXA[e]	335	3.2%	0.9%	296	3.3%	0.9%
SHV	99	0.9%	7.5%	91	1.0%	7.7%
TEM	6	0.1%	0.5%	3	<0.1%	0.3%
Carbapenemase	**114**	**1.1%**	**8.4%**	**86**	**0.9%**	**6.9%**
KPC	109	1.0%	8.3%	81	0.9%	6.9%
SME-4	2	<0.1%	0.2%	2	<0.1%	0.2%
NMC-A				2	<0.1%	0.2%
OXA-232	1	<0.1%	0.1%	1	<0.1%	0.1%
NDM-1	1	<0.1%	0.1%			
IMP-27	1	<0.1%	0.1%			
Transferable AmpC	**75**	**0.7%**	**5.5%**	**92**	**1.0%**	**7.4%**
CMY-2-like	52	0.5%	3.9%	78	0.9%	6.6%
DHA	15	0.1%	1.1%	8	0.1%	0.7%
FOX	8	0.1%	0.6%	8	0.1%	0.7%

[a] Total number of isolates included in the INFORM Program.
[b] Number of isolates carrying the gene.
[c] Percentage of the total number of isolates included in the program.
[d] Percentage of the total number of isolates screened for β-lactamase.
[e] All OXA-1/30 or OXA-1/30-like, except for 1 OXA-4 *K. pneumoniae* producer.
ESBLs, extended-spectrum β-lactamases.

Table 4 Occurrence of β-lactamase stratified by organism and year

Organism/β-lactamase class/β-lactamase gene (total no. 2016/2017)[a]	2016			2017		
	No.[b]	% of total[c]	% of screened[d]	No.[b]	% of total[c]	% of screened[d]
***E. coli* (3,751/3,421)[a]**						
ESBLs						
CTX-M	478	12.7%	85.7%	480	14.0%	81.8%
CTX-M-1 group	335	8.9%	60.0%	329	9.6%	56.0%
CTX-M-9 group	144	3.8%	25.8%	156	4.6%	26.6%
OXA[e]	200	5.3%	35.8%	176	5.1%	30.0%
SHV	5	0.1%	0.9%	8	0.2%	1.4%
TEM	4	0.1%	0.7%	2	0.1%	0.3%
Carbapenemase						
KPC	4	0.1%	0.7%	6	0.2%	1.0%
Transferable AmpC	48	1.3%	8.6%	73	2.1%	12.4%
CMY-2-like	45	1.2%	8.1%	72	2.1%	12.3%
DHA	2	0.1%	0.4%	3	0.1%	0.5%
FOX	1	<0.1%	0.2%			
Narrow spectrum						
OXA	2	0.1%	0.4%	1	<0.1%	0.2%
SHV	3	0.1%	0.5%	1	<0.1%	0.2%
TEM	203	5.4%	36.4%	197	5.8%	33.6%
Spectrum undetermined						
OXA				2	0.1%	0.3%
No β-lactamase detected	46	1.2%	5.1%	20	0.6%	5.4%
***K. pneumoniae* (2,201/1,834)[a]**						
ESBLs						
CTX-M	172	7.8%	58.9%	160	8.7%	61.3%
CTX-M-1 group	161	7.3%	55.1%	152	8.3%	58.2%
CTX-M-2 group	1	<0.1%	0.3%	1	0.1%	0.4%
CTX-M-9 group	11	0.5%	3.8%	7	0.4%	2.7%
OXA[e]	109	4.9%	37.3%	101	5.5%	38.7%
SHV	57	2.6%	19.5	61	3.3%	23.4%
TEM	2	0.1%	0.7%	2	0.1%	0.8%
Carbapenemase						
KPC	74	3.4%	25.3%	41	2.2%	15.7%
OXA-48-like	1	<0.1%	0.3%	1	0.1%	0.4%
Transferable AmpC						
CMY-2-like	4	0.2%	1.4%	4	0.2%	1.5%
DHA	8	0.4%	2.7%	3	0.2%	1.1%
FOX	3	0.1%	1.0%	3	0.2%	1.6%
Narrow spectrum						
CARB	2	0.1%	0.7%	4	0.2%	1.5%
OXA	52	2.4%	17.8%	19	1.0%	7.3%
SHV	229	10.4%	78.4%	216	11.8%	82.8%
TEM	192	8.7%	65.8%	155	8.5%	59.4%
Spectrum undetermined						
LAP				4	0.2%	1.5%
LEN				4	0.2%	1.5%
No β-lactamase detected	1	<0.1%	0.3%	2	0.1%	0.8%
***E. cloacae* (1,021/911)[a]**						
ESBLs						
CTX-M	20	2.0%	7.9%	20	2.2%	8.8%
CTX-M-1 group	18	1.8%	7.1%	18	2.0%	7.9%
CTX-M-2 group	1	0.1%	0.4%			
CTX-M-9 group	1	0.1%	0.4%	2	0.2%	0.9%
OXA[e]	13	1.3%	5.2%	12	1.3%	5.3%
SHV	35	3.4%	13.9%	33	3.6%	14.5%
TEM	2	0.2%	0.8%	1	0.1%	0.4%
Carbapenemase						
KPC	12	1.2%	4.8%	16	1.8%	7.0%
NDM-1	1	0.1%	0.4%			
NMC				2	0.2%	0.9%
Transferable AmpC						
CMY-2-like	1	0.1%	0.4%			
DHA	4	0.4%	1.6%	2	0.2%	0.9%
FOX				3	0.3%	1.3%
Narrow spectrum						
CARB				4	0.4%	1.8%
OXA	13	1.3%	5.2%	7	0.8%	3.1%
TEM	54	5.3%	21.4%	46	5.0%	20.2%
SHV				1	0.1%	0.4%
Spectrum undetermined						
LAP				4	0.4%	1.8%
OXA	1	0.1%	0.4%			
CMH				8	0.9%	3.5%
No β-lactamase detected	174	17.0%	69.0%	144	15.8%	63.2%

[a] Total number of isolates included in the INFORM Program.
[b] Number of isolates carrying the gene.
[c] Percentage of the total number of isolates included in the program.
[d] Percentage of the total number of isolates screened for β-lactamase.
[e] All OXA-1/30 or OXA-1/30-like, except for 1 OXA-4 *K. pneumoniae* producer.
ESBLs, extended-spectrum β-lactamases.

Table 5 Antimicrobial activity of ceftazidime-avibactam tested against the main groups of β-lactamase-producing isolates

Organism/organism group (no. of isolates)	No. and cumulative % of isolates inhibited at MIC (mg/L) of:													MIC_{50}	MIC_{90}
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32		
ESBL producers without carbapenemases															
2016 (759)	38	26	81	273	220	86	26	9						0.12	0.5
	5.0	8.4	19.1	55.1	84.1	95.4	98.8	100.0							
2017 (743)	21	7	60	273	245	97	28	10	2					0.25	0.5
	2.8	3.8	11.8	48.6	81.6	94.6	98.4	99.7	100.0						
CTX-M producers without carbapenemases															
2016 (669)	38	23	76	248	201	66	14	3						0.12	0.5
	5.7	9.1	20.5	57.5	87.6	97.5	99.6	100.0							
2017 (656)	18	6	53	251	223	77	18	8	2					0.12	0.5
	2.7	3.7	11.7	50.0	84.0	95.7	98.5	99.7	100.0						
OXA producers without carbapenemases[e]															
2016 (335)	24	11	37	113	101	32	13	4						0.12	0.5
	7.2	10.4	21.5	55.2	85.4	94.9	98.8	100.0							
2017 (296)	6	2	17	99	101	49	13	7	2					0.25	0.5
	2.0	2.7	8.4	41.9	76.0	92.6	97.0	99.3	100.0						
SHV-ESBL producers without carbapenemases															
2016 (87)	0	3	5	25	20	19	10	5						0.25	1
	0.0	3.4	9.2	37.9	60.9	82.8	94.3	100.0							
2017 (97)	2	1	8	23	24	27	9	3						0.25	1
	2.1	3.1	11.3	35.1	59.8	87.6	96.9	100.0							
Plasmidic AmpC producers without carbapenemases															
2016 (69)	3	3	4	21	20	9	3	4	1	1				0.25	1
	4.3	8.7	14.5	44.9	73.9	87.0	91.3	97.1	98.6	100.0					
2017 (91)	3	3	4	23	33	17	8							0.25	0.5
	3.3	6.6	11.0	36.3	72.5	91.2	100.0								
Carbapenemase producers															
2016 (109)	4	2	1	7	19	29	24	11	10	0	1	0	1	0.5	4
	3.7	5.5	6.4	12.8	30.3	56.9	78.9	89.0	98.2	98.2	99.1	99.1	100.0		
2017 (83)	2	0	2	2	9	14	27	21	5	0	0	1		1	2
	2.4	2.4	4.8	7.2	18.1	34.9	67.5	92.8	98.8	98.8	98.8	100.0			
Serine carbapenemase producers															
2016 (107)	4	2	1	7	19	29	24	11	9	0	1			0.5	2
	3.7	5.6	6.5	13.1	30.8	57.9	80.4	90.7	99.1	99.1	100.0				
2017 (80)	2	0	2	2	9	13	26	20	5	0	0	1		1	2
	2.5	2.5	5.0	7.5	18.8	35.0	67.5	92.5	98.8	98.8	98.8	100.0			

[a] Greater than the highest concentration tested.
[b] All OXA-1/30 or OXA-1/30-like, except for 1 OXA-4 *K. pneumoniae* producer.
ESBL, extended-spectrum β-lactamase.

Table 6 *Enterobacterales* isolates with ceftazidime-avibactam MIC values >8 mg/L (INFORM Program, 2016 and 2017)

Year / Organism	Location	Infection type	Resistance mechanism	MIC (mg/L)					
				CAZ-AVI	C-T	MEM	AMK	TIG	COL
2016									
E. cloacae	New York	SSSI	KPC-4, ↓OmpF	16	>32	4	1	2	0.12
Providencia stuartii	North Carolina	UTI	Unknown	16	16	0.06	1	2	>8
Providencia stuartii	Arizona	Pneumonia	CMY-2, cAmpC	16	>32	0.12	1	2	>8
E. cloacae	Louisiana	UTI	NDM-1, DHA-1, OXA-9	>32	>32	32	>3	0.5	0.12
2017									
S. marcescens	New York	Pneumonia	KPC-3, OXA-9, OXA-10, cAmpC	32	>16	>32	32	2	>8

Abbreviations: CAZ-AVI, ceftazidime-avibactam; C-T, ceftolozane-tazobactam; MEM, meropenem; AMK, amikacin; TIG, tigecycline; COL, colistin; SSSI, skin and skin structure infection; UTI, urinary tract infection; cAmpC, overexpression of chromosomal AmpC.

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