Table of Contents
1. Description ........................................................................................................................................ 3
2. Legend ............................................................................................................................................ 3
3. Literature Summary of Resistance Genes ......................................................................................... 3
   3.1. AAC (aac(3)-II(a-d), aac(3)-IV, aac(6’)-Ib/Ib-cr) ........................................................................ 3
   3.1.2. aac(3)-II(a-d) ........................................................................................................................ 3
   3.1.3. aac(3)-IV ............................................................................................................................... 5
   3.1.3. aac(6’)-Ib/Ib-cr ................................................................................................................... 6
   3.2. AAD (aadA3/A8) ....................................................................................................................... 7
   3.3. ANT (ant(2")-Ia) .................................................................................................................... 8
   3.4. APH (aph(4)-la) ...................................................................................................................... 8
   3.5. armA ....................................................................................................................................... 9
   3.6. CMY (cmy-2 and cmy-41) ..................................................................................................... 11
   3.7. CTX-M-1, CTX-M-2, CTX-M-9 ............................................................................................. 12
   3.8. DFR (dfrA5 and dfrA17) ......................................................................................................... 13
   3.9. DHA ....................................................................................................................................... 14
   3.10. GyrA – Mutant ....................................................................................................................... 14
   3.11. IMP (IMP-1, IMP-2, IMP-5) ............................................................................................... 15
   3.12. KPC ....................................................................................................................................... 16
   3.13. MCR-1 ................................................................................................................................... 17
   3.14. NDM ..................................................................................................................................... 17
   3.15. OXA-1, OXA-9, OXA-48 ...................................................................................................... 18
   3.16. PER ....................................................................................................................................... 19
   3.17. RMT (rmtB and rmtF) .......................................................................................................... 20
   3.18. SHV (shv-G156 (WT), shv-G156D) .................................................................................... 21
   3.19. sul1 and sul2 ...................................................................................................................... 23
   3.20. TEM (tem-E104 (WT) and tem-E104K) ............................................................................... 24
   3.21. vanA .................................................................................................................................... 25
   3.22. VIM (VIM-1, VIM-2, VIM-5, VIM-13) ............................................................................... 25
   3.23. VEB ..................................................................................................................................... 27
4. OpGen Headquarters Location ........................................................................................................ 28
5. Technical Assistance ....................................................................................................................... 28
1. Description

The Acuitas AMR Gene Panel Electronic User Guide (EUG) Literature Summary lists publications about the antimicrobial resistance genes reported by the Acuitas AMR Gene Panel.

2. Legend

Description of headings.

1. **Mechanistic Study**: Characterization of antibiotic resistance gene sequence or function (substrate specificity). Generally, first reported instance.

2. **Molecular Epi**: Clinical isolate screen for genes conferring antibiotic resistance.

   Method of antibiotic resistance gene detection:
   - PCR = polymerase chain reaction
   - WGS = whole genome sequencing
   - DNA hybridization
   - LAMP = Loop mediated isothermal amplification
   - Biochemical = evaluation of purified protein

3. **AST Study**: Novel antibiotic screened for activity against clinical isolates with antibiotic resistance genes.

4. **Isolate Study**: One or two clinical isolates characterized in depth for antibiotic resistance genes.


---

**Note**

Literature references indicated by square parentheses [ ].

Round parentheses ( ) within literature references indicate number of positive isolates or resistance genes in study. For example, “14 *E. coli* isolates harbored aac(3)-II variants: aac(3)-11d (1), aac(3)-11e (13)”.

---

3. Literature Summary of Resistance Genes

3.1. AAC (aac(3)-III(a-d), aac(3)-IV, aac(6')-Ib/Ib-cr)

   3.1.1. aac(3)-II(a-d)

   AAC(3)-II is also called aacC2, a major plasmid-mediated aminoglycoside modifying enzyme. First identified on a plasmid designated R factor 176 in *Klebsiella* which can transfer gentamicin-, tobramycin- and kanamycin-resistance to *Escherichia coli*. Enzyme confers resistance by 3-N enzymatic acetylation of gentamicin, tobramycin and kanamycin [1]. AAC(3)-II has been reported in *E. coli* [2-4, 6, 8], *K. pneumoniae* [2, 4, 8], *Proteus mirabilis* [8], *Pseudomonas aeruginosa* [5] and other *Enterobacteriaceae* [7, 8]. aac(3)-II(a-d) assay detects four gene subtypes, aac(3)-IIa, c-e.

Mechanistic Study: Initial Characterization of the aac(3)-II aminoglycoside modifying enzyme where the aac(3)-II gene was transferred to a susceptible *E. coli* strain and substrate aminoglycosides specificity determined.


Molecular Epi *E. coli, K. pneumoniae (WGS)*: Antibiotic resistance genes from 74 *E. coli* and 69 *K. pneumoniae* blood culture isolates were sequenced and analyzed for known resistance genes. 14 *E. coli* isolates harbored aac(3)-11d (1), aac(3)-11e (13). 23 *K. pneumoniae* isolates harbored aac(3)-IIa (1), aac(3)-IIIa (3) and aac(3)-Ile (19).


Molecular Epi *E. coli (PCR)*: 205 gentamicin-resistant *E. coli* clinical isolates screened for various aminoglycoside resistance genes including aac(3)-II (162).


Molecular Epi *E. coli, K. pneumoniae (PCR)*: clinical isolates of *E. coli* and *K. pneumoniae* screened for antibiotic resistance genes including aac(3)-IIa: *E. coli* (40 of 65), *K. pneumoniae* (20 of 23).


Molecular Epi *P. aeruginosa (PCR)*: 80 *P. aeruginosa* clinical isolates screened for various antibiotic resistance genes including 7 gentamicin-resistant isolates screened for aac(3)-II (5).


Molecular Epi *E. coli (PCR)*: 257 amoxicillin/clavulanate-resistant *E. coli* isolates screened for aminoglycoside resistance genes including aac(3)-IIa (23).


Molecular Epi *Enterobacteriaceae (PCR)*: 40 clinical isolates of *E. cloacae* screened for aminoglycoside resistance genes including aac(3)-II (11).

AST Study *E. coli, K. pneumoniae, P. mirabilis, Enterobacteriaceae* (PCR): 788 *Enterobacteriaceae* clinical isolates screened for common AMR genes including aac(3)-IIa: *E. coli* (175), *K. pneumoniae* (89), *K. oxytoca* (2), *P. mirabilis* (2), *E. cloacae* (1) and *E. aerogenes* (1).

### 3.1.2. aac(3)-IV

AAC(3)-IV is a plasmid-mediated aminoglycoside modifying enzyme (9). Enzyme has been detected in *E. coli* [8, 10, 11, 15] and *K. pneumoniae* [8, 11, 12], *P. mirabilis* [13] and *P. aeruginosa* [14]. aac(3)-IV assay detects gene subtype aac(3)-IV.

Ref Error! Reference source not found.. **AST Study *E. coli, K. pneumoniae (PCR):*** aac(3)-IVa: *K. pneumoniae* (22), *E. coli* (1).


   **Mechanistic study:** Substrate specificity of aac(3)-IV assessed with purified protein.


   **Molecular Epi *E. coli (DNA Hybridization):*** 26 gentamicin-resistant *E. coli* clinical isolates screened for aac(3)-IV (7).


   **Molecular Epi *E. coli, K. pneumoniae (DNA Hybridization):*** 22 gentamicin-resistant *E. coli* and 5 gentamicin-resistant *K. pneumoniae* clinical isolates screened for aac(3)-IV and detected in *E. coli* (5) and *K. pneumoniae* (1).


   **AST Study *K. pneumoniae (PCR):*** 50 carbapenem-resistant *K. pneumoniae* clinical isolates tested for efficacy with a novel aminoglycoside, and isolates screened for aminoglycoside resistance genes including aac(3)-IV (19).


   **Molecular Epi *P. mirabilis (PCR):*** 14 EBSL-producing *P. mirabilis* clinical isolates screened for various antibiotic resistance genes including aac(3)-IV (10).


   **Molecular Epi *P. aeruginosa (PCR):*** 122 *P. aeruginosa* clinical isolates screened for common aminoglycoside modifying enzymes including aac(3)-IV (13).

**Molecular Epi E. coli (PCR):** 122 clinical isolates of *E. coli* characterized for common resistance genes including aac(3)-IV (83).

### 3.1.3. aac(6′)-lb/lb-cr

AAC(6′)-lb is an aminoglycoside 6′-N-acetyltransferase. aac(6′)-lb-cr is a variant of aac(6′)-lb which also confers resistance to fluoroquinolone. Enzyme reduces activity of ciprofloxacin by N-acetylation at amino nitrogen on piperazinyl substituent. Two amino acid changes in aac(6′)-lb-cr, Trp102Arg and Asp179Tyr, are together necessary to acetylate ciprofloxacin [16]. aac(6′)-lb and aac(6′)-lb-cr detected in *E. coli* [8, 17, 18], *K. pneumoniae* [8, 12, 18], *P. aeruginosa* [19], *P. mirabilis* [8, 20, 21] and other *Enterobacteriaceae* [8, 21]. aac(6′)-lb/lb-cr assay detects gene subtypes aac(6′)-lb, aac(6′)lb-cr, aacA4, aacA4-8, ant(3′)-Ib-aac(6′)-Ild and aac(3)-lb-aac(6′)-lb.

Ref Error! Reference source not found.. **AST Study E. coli, K. pneumoniae, P. mirabilis, E nterobacteriaceae (PCR):** aac(6′)-lb renders resistance in *E. coli* (102), *K. pneumoniae* (154), *P. mirabilis* (3) and other *Enterobacteriaceae* *K. oxytoca* (6), *E. cloacae* (4).

Ref 12. **AST Study K. pneumoniae (PCR):** aac(6′)-1b (49).


**Mechanistic Study:** Initial characterization of aac(6′)-1b-cr conferring fluoroquinolone resistance.


**Molecular Epi E. coli (PCR):** 539 ciprofloxacin- and/or tobramycin-resistant *E. coli* isolates from urinary tract infections screened for aac(6′)-lb-cr (59).


**Molecular Epi E. coli, K. pneumoniae (PCR):** 39 of 60 *E. coli* and 33 of 60 *K. pneumoniae* ESBL-producing clinical isolates contained aac(6′)-1b. Of these 25 of 39 and 19 of 33 were aac(6′)-1b-cr variant.


**Molecular Epi P. aeruginosa (PCR):** 122 multidrug-resistant *P. aeruginosa* clinical isolates screened for aminoglycoside and carbapenem resistance determinants including aac(6′)-lb (50).

**Molecular Epi P. mirabilis (PCR):** aac(6’)-Ib gene detected in 5 of 7 of aminoglycoside-resistant *P. mirabilis* clinical isolates.


**Molecular Epi P. mirabilis, Enterobacteriaceae (PCR):** Clinical isolates of *P. mirabilis, P. vulgaris* and *M. morganii* screened for various aminoglycoside and carbapenem resistance genes. aac(6’)-Ib: *P. mirabilis* (7) and other *Enterobacteriaceae P. vulgaris* (3) and *M. morganii* (1). aac(6’)-Ib-cr: *P. mirabilis* (1).

### 3.2. AAD (aadA3/A8)

aadA is a streptomycin/spectinomycin adenyltransferase, an aminoglycoside 3’(9)-O-nucleotidyl-transf erase (aad(3’)). *E. coli* encoding aadA gene identified by insertion/ deletion mutation and gene sequence determined [23]. Enzyme reported in Gram-positive and -negative bacteria: *E. coli* [22,23], *K. pneumoniae* [24, 26], *P. aeruginosa* [25, 26], *P. mirabilis* [21, 27], *Enterococcus faecalis* [26, 28] and other *Enterobacteriaceae* [21, 24, 26]. AAD assay detects gene subtypes aadA2, aadA3, aadA8 and aadA8b.

Ref Error! Reference source not found.. **Molecular Epi P. mirabilis, Enterobacteriaceae (PCR):** aadA2: *P. mirabilis* (4) and other *Enterobacteriaceae P. vulgaris* (1) and *M. morganii* (1).


**Molecular Epi E. coli (PCR):** 70 Enteropathogenic *E. coli* clinical isolates screened for aadA2 (2).


**Mechanistic Study:** Nucleotide sequence of aadA deduced.


**Molecular Epi K. pneumoniae Enterobacteriaceae (PCR):** antibiotic resistance genes found in class 1 integrons characterized in a panel of clinical isolates including aadA2 in *K. pneumoniae* (72) and other *Enterobacteriaceae K. oxytoca* (5).


**Mechanistic Study:** First report of aadA2 gene in *P. aeruginosa*.


**Molecular Epi P. mirabilis (PCR):** 79 clinical isolates screened for antibiotic resistance genes found on class 1 integrons including aadA2 (57).


**Mechanistic Study:** *E. faecalis* aadA nucleotide sequences reported identical to *E. coli* variants.

### 3.3. ANT (ant(2")-Ia)

Adenylyltransferase ant(2")-Ia is one of most prevalent aminoglycoside resistance enzymes in Gram-negative pathogens. Enzyme confers resistance to gentamicin, tobramycin and kanamycin. ant(2")-Ia reported in *P. aeruginosa* [29, 30], *E. coli* [6, 8], *K. pneumoniae* [8, 12], *P. mirabilis* [8, 21] and other *Enterobacteriaceae* [8, 21]. ANT assay detects gene subtype ant(2")-Ia (aadB).

Ref 6. **Molecular Epi E. coli (PCR):** ant(2")-Ia (29).

Ref Error! Reference source not found.. **AST Study E. coli, K. pneumoniae, P. mirabilis, Enterobacteriaceae (PCR):** ant(2")-Ia: *E. coli* (11), *K. pneumonia* (14) *P. mirabilis* (8), and other *Enterobacteriaceae K. oxytoca* (2), *E. cloacae* (1).

Ref 12. **AST Study K. pneumoniae (PCR):** ant(2")-Ia (1).

Ref Error! Reference source not found.. **Molecular Epi P. mirabilis, Enterobacteriaceae (PCR):** ant(2")-Ia: *P. mirabilis* (1) and other *Enterobacteriaceae P. vulgaris* (2) and *M. morganii* (1).


**Molecular Epi P. aeruginosa (PCR):** 98 *P. aeruginosa* clinical isolates partitioned into 2 clusters with ant(2")-Ia detected in 1 cluster (19).


**Molecular Epi P. aeruginosa (PCR):** 200 Gram-negative clinical isolates including 52 *P. aeruginosa* screened for aminoglycoside resistance genes including ant(2")-1a (12).

### 3.4. APH (aph(4)-Ia)

APH(4)-Ia is an aminoglycoside phosphotransferase conferring resistance to the atypical aminoglycoside antibiotic hygromycin B (hygB) by phosphorylation of 4 hydroxyl position of cyclitol ring (hyosamine) [31].
aph(4')-Ia detected in *E. coli* [31, 32], *P. mirabilis* [33] and *K. pneumoniae* [34]. APH assay detects gene subtype aph(4)-Ia.


**Mechanistic Study:** Initial characterization of aph(4)-Ia gene.


**Isolate Characterization E. coli (WGS):** Draft genome of 1 *E. coli* clinical isolate reported antibiotic resistance genes including aph(4)-Ia.


**Isolate Characterization P. mirabilis (WGS):** 2 *P. mirabilis* clinical isolates. Various antimicrobial resistance genes identified in both genomes including genes conferring aminoglycosides resistance: aph(4)-Ia (2).


**Isolate Characterization K. pneumoniae (WGS):** Antibiotic resistance genes reported in one clinical isolate of *K. pneumoniae* including aph(4)-1a (1).

### 3.5. armA

armA is a plasmid-borne 16S rRNA methyltransferase conferring high-level resistance to aminoglycosides. armA reported in *E. coli* [35, 36, 37, 43], *K. pneumoniae* [37, 38, 41, 43, 46], *P. aeruginosa* [30, 39, 42, 43] and *P. mirabilis* [21, 40, 43] and other *Enterobacteriaceae* [21, 41, 43]. armA assay detects armA gene.

Ref Error! Reference source not found.. **Molecular Epi P. mirabilis Enterobacteriaceae (PCR):** armA: *P. m irabilis* (1) and other *Enterobacteriaceae P. vulgaris* (1).

Ref 30. **Molecular Epi P. aeruginosa (PCR):** armA (1).

Ref 46. **Isolate Study K. pneumoniae:** armA (1).


**Mechanistic Study:** First characterization of armA gene including nucleotide sequence.


**Molecular Epi E. coli (WGS):** 38 carbapenem-resistant isolates analyzed for NDM variants and aminoglycoside resistance determinants. 33 of 38 isolates harbored 16S rRNA methylase-encoding genes including armA (14).

**Molecular Epi E. coli, K. pneumoniae (PCR):** 28 *E. coli* and 53 *K. pneumoniae* clinical isolates with resistance amikacin screened for armA: *E. coli* (12) and *K. pneumoniae* (16).


**Molecular Epi K. pneumoniae (PCR):** 17 amikacin/kanamicin-resistant *K. pneumoniae* isolates screened for genes conferring resistance to aminoglycosides including armA (17).


**Molecular Epi P. aeruginosa (PCR):** 92 clinical isolates of *P. aeruginosa* screened for aminoglycoside resistance genes including armA (1).


**Molecular Epi P. mirabilis (PCR):** 44 EBSL-producing *P. mirabilis* characterized for armA (3).


**Molecular Epi K. pneumoniae, Enterobacteriaceae (PCR):** aminoglycoside-resistance clinical isolates of Gram-negative bacilli screened for armA gene. armA was detected in clinical isolates of *E. coli* (3), *K. pneumoniae* (14) and other *Enterobacteriaceae* C. freundii (1), *E. cloacae* (2), *S. marcescens* (4).


**Molecular Epi P. aeruginosa (PCR):** 33 aminoglycoside-resistant *P. aeruginosa* clinical isolates harbouring NDM-1 were screened for aminoglycoside resistance by 16s rRNA methyltransferases including armA (10).


**Molecular Epi E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, Enterobacteriaceae (PCR):** 217 amikacin/gentamicin-resistant Gram-negative clinical isolates screened for aminoglycoside resistance genes including armA: *E. coli* (1 of 9), *K. pneumoniae* (26 of 36), *P. mirabilis* (3 of 7), *P. aeruginosa* (2 of 5) and other *Enterobacteriaceae* S. marcescens (1 of 1).
3.6. CMY (cmy-2 and cmy-41)

CMY is a plasmid-mediated AmpC β-lactamase conferring resistance to extended-spectrum beta-lactams including cephemycins (cephemycinase, CMY). CMY detected in *K. pneumoniae* [44-46, 53], *E. coli* [44, 47-48, 52], *P. mirabilis* [49-50] and *P. aeruginosa* [51]. Test has two assays, CMY-2 and CMY-41, each of which detect several gene subtypes.


**Isolate Study E. coli, K. pneumoniae (WGS):** CMY-2 detected in two clinical isolates: *E. coli* (1) and *K. pneumoniae* (1).


**Molecular Epi K. pneumoniae (PCR):** 32 clinical isolates of *K. pneumoniae* screened for ampC variants including CMY-2 (9).


**Isolate Study K. pneumoniae (WGS):** *K. pneumoniae* clinical isolate harbored CMY-16.


**Isolate Study E. coli (WGS):** 70 clinical isolates of *E. coli* screened for antibiotic resistance genes including CMY-2 (11).


**Isolate Study E. coli (WGS):** a clinical isolate of *E. coli* harbored CMY-2.


**Mechanistic Study P. mirabilis:** First report of CMY-16 variant.


**Molecular Epi P. mirabilis (PCR):** 19 clinical isolates of *P. mirabilis* screened for CMY-2 (8).

**Molecular Epi *P. aeruginosa (PCR)*:** 329 clinical isolates of *P. aeruginosa* screened for ampC variants including CMY-2 (48).


**Molecular Epi *E. coli (PCR)*:** 1,210 clinical isolates of *E. coli* screened for CMY-2 (10).


**Molecular Epi *K. pneumoniae (PCR)*:** 260 *K. pneumoniae* clinical isolates screened for ampC variants including CMY-2 (2) and CMY-10 (1).

### 3.7. CTX-M-1, CTX-M-2, CTX-M-9

CTX-M is a large family of chromosomal and plasmid-encoded extended-spectrum β-lactamases. CTX-M hydrolyzes cefotaxime and ceftazidime. CTX-M are common worldwide in clinical strains of *E. coli* [54, 55]. Also reported in *K. pneumoniae* [54, 55, 56], *P. mirabilis* [54, 55, 57], *P. aeruginosa* [58] and other *Enterobacteriaceae* [54, 55]. Test has three CTX-M assays: CTX-M-1, CTX-M-2 and CTX-M-9, each of which detects several gene subtypes.


**Review Article:** CTX-M beta lactamases were reviewed including molecular epi studies demonstrating CTX-M-1 in *E. coli*, CTX-M-2 in *E. coli*, *K. pneumoniae*, *P. mirabilis* and other *Enterobacteriaceae Salmonella typhimurium* and *Enterobacter spp.*


Molecular Epi *P. mirabilis* (DNA hybridization): 8 species of *Enterobacteriaceae* characterized for CTX-M variants including CTX-M-2 in *P. mirabilis* (1).


Isolate Study *P. aeruginosa*: clinical isolate of *P. aeruginosa* sequenced and the presence plasmid encoded CTX-M-2 described.

3.8. DFR (dfrA5 and dfrA17)

Some subtypes of dihydrofolate reductases (DHFR, encoded by *dfr* genes) confer resistance to trimethoprim. Among trimethoprim-resistant *dfr* genes, *dfrA17* is most common subtype in urinary isolates of *E. coli* in Korea [59]. *dfr* genes detected in *E. coli* [26, 59, 60, 61], *K. pneumoniae* [26, 61, 62], *P. mirabilis* [63], *E. faecalis* [26], *P. aeruginosa* [26] and other *Enterobacteriaceae* [26]. DFR assay detects genes *dfrA5* and *dfrA17*.


Molecular Epi *E. coli* (PCR): 421 trimethoprim-resistant clinical isolates of *E. coli* screened for *dfrA* variants including *dfrA5* (7) and *dfrA17* (56).


Molecular Epi *E. coli* (PCR): 77 trimethoprim-resistant clinical isolates of *E. coli* screened for *dfrA* variants including *dfrA5* (4) and *dfrA17* (27).


Molecular Epi *E. coli, K. pneumoniae* (PCR): 320 clinical isolates of *E. coli* and 54 clinical isolates of *K. pneumoniae* screened for *dfrA* variants including *dfrA5*: *E. coli* (52) and *K. pneumoniae* (7) and *dfrA17*: *E. coli* (82) and *K. pneumoniae* (1).


Molecular Epi *K. pneumoniae* (PCR): Antibiotic resistance genes in class 1 integrons characterized including *dfrA17* for 20 clinical isolates of *K. pneumoniae* (14).

**Molecular Epi *P. mirabilis* (PCR):** 108 clinical isolates of *P. mirabilis* characterized for antibiotic resistance genes including dfrA17 (7).

### 3.9. DHA

DHA is a plasmid-mediated AmpC β-lactamase (cephalosporinase) conferring resistance to extended-spectrum cephalosporins and cephemycins. DHA function demonstrated by transferring resistance to oximino-cephalosporins (ceftaxime and ceftazidime) and cephemycins (cefoxitin and moxalactam) by transforming *E. coli* with DHA plasmid from *Salmonella enteritidis* [64]. DHA gene detected in *E. coli* [47, 64], *K. pneumoniae* [45, 56, 65, 67] and *P. mirabilis* [68]. DHA assay detects several gene subtypes of DHA and blaMOR-2 (also plasmid-encoded ampC genes).

Ref 45. **Molecular Epi *K. pneumoniae* (PCR):** DHA-1 (20).
Ref 47. **Isolate Study *E. coli* (WGS):** DHA-1 (1).
Ref 56. **Molecular Epi *K. pneumoniae* (PCR):** DHA-1 (52).


**Isolate Study *E. coli*:** DHA-1 reported in 3 clinical isolates of *E. coli* (3).


**Molecular Epi *K. pneumoniae* (PCR):** 312 clinical isolates of *K. pneumoniae* characterized for ampC beta-lactamase genes including DHA-1 (312).


**Isolate Study *K. pneumoniae*:** First report of DHA-2 variant.


**Isolate Study *K. pneumoniae*:** First report of DHA-3 variant.


**Isolate Study *P. mirabilis*:** First report of DHA-1 in *P. mirabilis*.

### 3.10. GyraseA – Mutant

DNA gyrase introduces negative supercoils into DNA during DNA replication. Mutation of GyrA gene at Ser83 and/or Asp87 confers resistance to fluoroquinolones in *E. coli* [69, 70]. GyrA with mutation of Thr83 and/or Asp87 confers resistance to fluoroquinolones in *P. aeruginosa* [71]. *E. coli* gyrA-87-wild type assay detects codon GAC. *E. coli* gyrA-87-mutant assay detects Asp87Asn, Asp87Gly and Asp87Tyr. PA gyrA-83-wild type assay detects codon ACC. PA gyrA-83-mutant assay detects Thr83Ile.

**Mechanistic Study**: mutations conferring fluoroquinolone resistance in gyrA characterized from clinical isolates of *E. coli*.


**Mechanistic Study**: mutations conferring fluoroquinolone resistance in gyrA characterized from clinical isolates of *E. coli* and *K. pneumoniae*.


**Isolate Study P. aeruginosa**: Clinical isolate of *P. aeruginosa* responsible for clonal spread in hospital characterized for antibiotic resistance genes and demonstrated to harbour Thr83Ile mutation in GyrA.

3.11. IMP (IMP-1, IMP-2, IMP-5)

IMP is a large family of chromosomal and plasmid-borne integron metallo-β-lactamases (carbapenemases) with multiple gene subtypes. IMP-1 characterized and shown to confer resistance to all carbapenems, penicillins and cephalosporins. Resistance not eliminated with any β-lactamase inhibitor [72]. IMP identified in *K. pneumoniae* [72, 73], *P. aeruginosa* [74-76], *P. mirabilis* [77, 78], *E. coli* [79] and other *Enterobacteriaceae* [78]. IMP assay detects several gene subtypes across gene families IMP-1, IMP-2 and IMP-5.


**Mechanistic Study**: First report of IMP-1 in a clinical isolate of *K. pneumoniae* from Singapore.


**Mechanistic study**: First report of the IMP-8 in *K. pneumoniae*.


**Molecular Epi P. aeruginosa (PCR)**: 300 clinical isolate of *P. aeruginosa* screened for metallo-β-lactamase resistance genes including IMP-1 (10).


**Molecular Epi P. aeruginosa (WGS)**: 40 clinical isolates of *P. aeruginosa* with metallo-β-lactamases including IMP-15 (10) and IMP-26 (12) and IMP-51 (3).

**Molecular Epi P. aeruginosa (PCR):** 298 carbapenem non-susceptible *P. aeruginosa* isolates screened for metallo-β-lactamase resistance genes including IMP-15 (2).


**Isolate Study P. mirabilis:** 2 clinical isolates of *P. mirabilis* positive for IMP-27 (2).


**Isolate Study P. mirabilis, Enterobacteriaceae:** Plasmids of 2 clinical isolates possessed IMP-27: *P. mirabilis* (1) and other *Enterobacteriaceae Providencia rettgeri* (1).


**Molecular Epi E. coli (PCR):** 115 clinical isolates screened for β-lactam resistance genes including IMP-6 (3).

### 3.12. KPC

KPC is a large family of chromosomal and plasmid-mediated carbapenemases belonging to Ambler class A serine-β-lactamases (SBLs). KPC-1 identified in *K. pneumoniae*, cloned in *E. coli* and shown to confer resistance to imipenem, meropenem, extended-spectrum cephalosporins, and aztreonam [44, 80]. KPC has spread from *K. pneumoniae* to many species including *E. coli* [44, 81], *P. aeruginosa* [82] and *P. mirabilis* [83]. KPC assay detects several gene subtypes of kpc.

Ref 44. **Isolate Study E. coli, K. pneumoniae (WGS):** KPC-2: *E. coli* (1) and *K. pneumoniae* (1)


**Mechanistic Study:** First report of KPC-1 gene in *K. pneumoniae* including nucleotide sequence.


**Molecular Epi E. coli (WGS):** 43 of 45 clinical isolates of *E. coli* demonstrated to possess KPC-1.


**Isolate Study P. aeruginosa:** First report of KPC in clinical isolate of *P. aeruginosa*.

**Isolate Study P. mirabilis**: First report of a KPC in clinical isolate of *P. mirabilis*.

### 3.13. MCR-1

MCR is a plasmid-mediated phosphoethanolamine transferase enzyme, conferring resistance to polymyxin colistin. MCR genes found in *K. pneumoniae* [84, 86], *E. coli* [85, 86] and other *Enterobacteriaceae* [87]. MCR-1 assay detects mcr-1 gene.


**Molecular Epi K. pneumoniae (PCR)**: Clinical isolates of *K. pneumoniae* from 283 patients screened for mcr-1 (16).


**Molecular Epi E. coli (PCR)**: 144 *E. coli* isolates from blood stream infections screened for mcr-1 (3).


**AST Study E. coli, K. pneumoniae**: antimicrobial activity of resveratrol in combination with colistin in clinical isolates characterized to possess mcr-1: *E. coli* (5), *K. pneumoniae* (1).


**Molecular Epi Enterobacteriaceae (PCR)**: 58 nonduplicate Gram-negative bacillus clinical isolates screened for mcr-1 and detected in other *Enterobacteriaceae E. cloacae* isolates (2).

### 3.14. NDM

NDM (New Delhi metallo beta-lactamase) is a family of highly mobile plasmid-encoded metallo-β-lactamases (carbapenemases). Enzyme hydrolyzes broad range of β-lactams (e.g., ampicillin, penicillin, piperacillin, cefepime, cefotaxime, cefoxitin, ceftazidime, cefuroxime, cephalothin, faropenem, imipenem, meropenem) but not aztreonam. blaNDM-1 identified in *K. pneumoniae* [88]. NDM is monomeric and can hydrolyze all beta-lactams except aztreonam [88]. NDM detected in *K. pneumoniae* [46, 88, 89, 90], *E. coli* [36, 89,90], *P. aeruginosa* [91], *P. mirabilis* [92, 93] and other *Enterobacteriaceae* [89]. NDM assay detects several gene subtypes of ndm.

Ref 46. **Isolate Study K. pneumoniae**: NDM-1 (1).

Ref 36. **Molecular Epi E. coli (WGS)**: NDM-1 (15).


**Mechanistic Study**: First characterization of NDM-1 as a novel metallo-β-lactamase.

**Molecular Epi E. coli, K. pneumoniae, Enterobacteriaceae (PCR):** 24 carbapenem-resistance Enterobacteriaceae screened for NDM-1. 22 possessed the gene: Klebsiella spp. (10), E. coli (9) and other Enterobacteriaceae Enterobacter spp. (2) and M. morganii (1).


**Isolate Study E. coli, K. pneumoniae:** Patient urine specimen harbored K. pneumoniae and E. coli, both of which carried NDM-1 gene.


**Molecular Epi P. aeruginosa (PCR):** 7 clinical isolates of P. aeruginosa screened for NDM-1 (2).


**Isolate Study P. mirabilis:** Extensively drug resistant clinical isolate of P. mirabilis carried NDM-1.


**Isolate Study P. mirabilis:** clinical isolate harbored NDM-5.

### 3.15. OXA-1, OXA-9, OXA-48

OXA is a large family of chromosomal and plasmid-mediated oxacillinsases a class D β-lactamases. Resistance mechanism OXA-1 studied in E. coli with purified OXA-1 protein [94]. OXAs usually confer resistance to amino- and ureidopenicillin and possess high-level hydrolytic activity against cloxacillin, oxacillin and methicillin. OXAs reported in E. coli [94-97], P. aeruginosa [98], K. pneumoniae [99-102] and P. mirabilis [103]. Test has three OXA assays, OXA-1, OXA-9, OXA-48, which detect 5, 1 and 11 subtypes of OXA.


**Molecular Epi E. coli (PCR):** 24 clinical isolates of E. coli positive for OXA-1.


**Isolate Study E. coli:** First report of OXA-181 in E. coli.


**Molecular Epi E. coli (PCR):** OXA-48 variant OXA-244 characterized in 5 clinical isolates of E. coli.

Isolate Study *E. coli*: OXA-48 reported in a clinical isolate of *E. coli*.


Isolate Study *P. aeruginosa*: First report of OXA-198 in *P. aeruginosa*.


Isolate Study *K. pneumoniae*: 2 carbapenem-resistant clinical isolates of *K. pneumoniae* characterized for beta-lactam resistance genes including OXA-48 (2).


Isolate Study *K. pneumoniae*: Clinical isolate of *K. pneumoniae* in hospital outbreak carried OXA-1.


Mechanistic Study: characterization of the OXA-9 variant from *K. pneumoniae*.


Isolate Study *P. mirabilis*: Clinical isolate of *P. mirabilis* harboring OXA-48.

3.16. PER

PER is a family of plasmid-encoded β-lactamases which hydrolyzes penicillins, cefotaxime, ceftazidime and aztreonam, but not carbapenems or cefamycins. *P. aeruginosa* and *E. coli* transformed with recombinant plasmid harboring PER-1 confirmed extended-spectrum cephalosporin resistance of PER-1. PER reported in *P. aeruginosa* [104-105, 107], *K. pneumoniae* [55, 105-107], *E. coli* [105-107], *P. mirabilis* [107] and other *Enterobacteriaceae* [106]. PER assay detects six gene subtypes of per.

Ref Error! Reference source not found.. Molecular Epi *K. pneumoniae* (PCR): PER-1: *K. pneumoniae* (1).


Isolate Study *P. aeruginosa*: First report of PER β-lactamase.


Molecular Epi *E. coli, K. pneumoniae, Enterobacteriaceae* (PCR): 39 extended-spectrum cephalosporin-resistant isolates screened for β-lactam resistance genes including PER-2 in *E. coli* (1 of 5), *K. pneumoniae* (2 of 10), and other *Enterobacteriaceae* *E. aerogenes* (1 of 3) and *E. cloacae* (3 of 7).


Molecular Epi *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa* (PCR): 613 clinical isolate of Gram-negative bacteria screened for PER-1 and detected in *E. coli* (27), *K. pneumoniae* (1), *P. mirabilis* (1) and *P. aeruginosa* (15).

3.17. RMT (rmtB and rmtF)

RmtB and RmtF are plasmid-mediated 16S rRNA methyltransferases conferring resistance to aminoglycoside including kanamycins and gentamicins. RmtB detected in *E. coli* [37, 43], *K. pneumoniae* [37, 41, 43, 108, 110, 113], *P. mirabilis* [40, 43, 108], and *P. aeruginosa* [39, 43, 109]. rmtF gene first characterized in *K. pneumoniae* [111] and subsequently in *E. coli* [112], *K. pneumoniae* [112], *P. aeruginosa* [30, 39, 42, 109, 114] and other *Enterobacteriaceae* [112]. RMT assay detects rmtB and rmtF genes.

Ref 30. **Molecular Epi P. aeruginosa (PCR):** rmtF (1).

Ref **Error! Reference source not found.**. **Molecular Epi E. coli K. pneumoniae (PCR):** rmtB in *E. coli* (2) and *K. pneumoniae* (5).

Ref 39. **Molecular Epi P. aeruginosa (PCR):** rmtB (12) rmtF (6).

Ref 40. **Molecular Epi P. mirabilis (PCR):** rmtB (2).

Ref **Error! Reference source not found..** **Molecular Epi K. pneumoniae (PCR):** rmtB (1).

Ref **Error! Reference source not found..** **Molecular Epi P. aeruginosa (PCR):** rmtF (10).

Ref **Error! Reference source not found..**. **Molecular Epi E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa (PCR):** rmtB: *E. coli* (9), *K. pneumoniae* (36), *P. mirabilis* (7), *P. aeruginosa* (5).


**Molecular Epi K. pneumoniae, P mirabilis (PCR):** rmtB detected in 3 of 1534 clinical isolates of *K. pneumoniae* and 3 of 734 *P. mirabilis*.

Molecular Epi *P. aeruginosa* (WGS): 45 clinical isolates of *P. aeruginosa* screened for antibiotic resistance genes including rmtB (3) and rmtF2 (1).


Molecular Epi *K. pneumoniae* (WGS): 27 clinical isolates of *K. pneumoniae* screened for carbapenamase and aminoglycoside resistance genes through 16s methylation including rmtB (17).


Initial Characterization *K. pneumoniae*: Initial characterization of rmtF 16s rRNA methyltransferase by transferring rmtF plasmid and aminoglycoside resistance from *K. pneumoniae* to susceptible strain of *E. coli*.


Molecular Epi *E. coli, K. pneumoniae and other Enterobacteriaceae* (PCR): 140 aminoglycoside-resistant clinical isolates of *Enterobacteriaceae* screened for rmtF: *K. pneumoniae* (17), *E. coli* (10) and other *Enterobacteriaceae* *C. freundii* (3), *E. cloacae* (4).


Molecular Epi *K. pneumoniae* (PCR): 78 clinical isolates screened for antibiotic resistance genes including rmtB (14).


Molecular Epi *P. aeruginosa* (PCR): antibiotic resistance genes present in 7 VIM-producing *P. aeruginosa* clinical isolates characterized including rmtB (1).

3.18. SHV (shv-G156 (WT), shv-G156D)

SHV is a large family of chromosomal and plasmid-encoded β-lactamases. Wild-type SHV confers resistance to penicillins and cephalosporins with a narrow spectrum activity and can be blocked by β-lactamase inhibitors [115]. SHV harboring mutations at G156D, G238S, and/or E240K becomes SHV ESBL (extended spectrum β-lactamases) conferring resistance to broad array of penicillins and cephalosporins. Some activity against carbapenems (e.g., meropenem) [116, 117]. SHV detected in *K. pneumoniae* [2, 55, 110, 115, 118, 121], *E. coli* [55, 119, 121], *P. aeruginosa* [120] and *P. mirabilis* [55, 121]. SHV-G156 (wild type) assay detects several gene subtypes while the SHV-G156D assay detects 5 gene subtypes associated with ESBL phenotype.

Ref Error! Reference source not found.. Molecular Epi *E. coli, K. pneumoniae, P. mirabilis* (PCR): SHV-like: *E. coli* (6), *K. pneumoniae* (103), *P. mirabilis* (2).


**Molecular Epi *K. pneumoniae* (PCR):** 97 clinical isolates of *K. pneumoniae* screened for SHV-1 (74).


**Mechanistic Study:** Nucleotide sequences of SVH-48 and SHV-105 variants described.


**Mechanistic Study:** SHV-5 described from *K. pneumoniae*.


**Mechanistic Study:** SHV-3 reported in *K. pneumoniae*.


**Molecular Epi *E. coli* (PCR):** 109 clinical isolates of *E. coli* screened for β-lactam resistance genes including SHV (9).


**Molecular Epi *P. aeruginosa* (PCR):** 123 clinical isolates of *P. aeruginosa* screened for SHV (13).


**Molecular Epi *E. coli, K. pneumoniae, P. mirabilis* (PCR):** Clinical isolates of *Enterobacteriaceae* screened for β-lactam resistance genes including SHV-1 which were detected in *E. coli* (60 of 98), *K. pneumoniae* (131 of 153) and *P. mirabilis* (14 of 21).
3.19. sul1 and sul2

sul1 and sul2 are plasmid-mediated dihydropteroate synthase (DHPS) conferring resistance to sulfonamide in Gram-negative bacilli [122,123]. sul1 and sul2 reported in *E. coli* [47, 122, 124, 125], *P. aeruginosa* [126], *P. mirabilis* [127] and *K. pneumoniae* [46, 128]. Separate assays detect sul1 and sul2 genes.

Ref 46. **Isolate Study *K. pneumoniae***: sul1(1), sul2(1).

Ref 47. **Isolate Study *E. coli* (WGS)**: sul1, sul2 or sul3 (48).


**Molecular Epi *E. coli* (PCR)**: sul1 characterized in clinical isolates of *E. coli* in 1991 and 1999: 143 of 360 isolates [39.7%] in 1991; 165 of 359 [46.0%] in 1999.


**Mechanistic Study**: nucleotide sequence of sul2 reported.


**Isolate Study *E. coli***: *E. coli* isolates of strain type 1642 characterized and demonstrated to possess plasmid carrying both sul1 and sul2.


**Molecular Epi *E. coli* (PCR)**: 26 *E. coli* clinical isolates screened for sul genes: sul1 (6), sul2 (12) and sul1+sul2 (8).


**Isolate Study *P. aeruginosa***: sul1 reported while characterizing antibiotic resistance genes in *P. aeruginosa*.


**Isolate Study *P. mirabilis***: Clinical isolate of *P. mirabilis* reported to carry sul2.


*Isolate Study K. pneumoniae:* sul1 reported while characterizing VIM-1 containing plasmid in clinical isolate of *K. pneumoniae.*

3.20. TEM (tem-E104 (WT) and tem-E104K)

TEM is a large family of chromosomal and plasmid-encoded β-lactamases. Wild-type TEM confers resistance to penicillins and cephalosporins with narrow spectrum activity. TEM activity blocked by β-lactamase inhibitors [129, 130]. TEM harboring mutations at E104K, R164H/C/S, G238S and/or E240K becomes TEM ESBL (extended spectrum β-lactamases) conferring resistance to a broad array of penicillins and cephalosporins [131, 132]. TEM detected in *E. coli* [44, 48, 55, 110, 133], *K. pneumoniae* [44, 55, 110, 133], *P. aeruginosa* [134] *P. mirabilis* [55, 135] and other *Enterobacteriaceae* [55, 130]. Assay detects several wild type and ESBL subtypes of TEM.

Ref 44. **Isolate Study E. coli and K. pneumoniae (WGS):** TEM-1: *E. coli* (1) and *K. pneumoniae* (1)

Ref 48. **Isolate Study E. coli (WGS):** TEM-1 (1).

Ref Error! Reference source not found.. Molecular Epi *E. coli, K. pneumoniae, P. mirabilis* and *Enterobacteriaceae* (PCR): TEM-like: *E. coli* (12), *K. pneumoniae* (82), *P. mirabilis* (2) and other *Enterobacteriaceae* *E. cloacae* (98).

Ref Error! Reference source not found.. Molecular Epi *K. pneumoniae* (WGS): TEM-1 (25).


**Mechanistic Study:** TEM-1 activity inhibited by novel β-lactamase inhibitor.


**Molecular Epi E. coli, P. mirabilis, Enterobacteriaceae (Biochemical)** TEM-1: *E. coli* (73 of 110), *P. mirabilis* (1 of 7) and other *Enterobacteriaceae* *E. cloacae* (1 of 5). *M. morganii* (1 of 2).


**Mechanistic Study:** Characterized substrate specificity of TEM-1 with mutations at position 104.


**Mechanistic Study:** Characterized substrate specificity of TEM-1 with mutations at positions 238 to 241.

Molecular Epi *E. coli, K. pneumoniae* (PCR): 170 *E. coli* isolates and 70 *K. pneumoniae* isolates characterized for beta-lactamase resistance genes including TEM-variants in *E. coli* (17) and *K. pneumoniae* (14).


Molecular Epi *P. aeruginosa* (PCR): 116 *P. aeruginosa* clinical isolates screened for β-lactamase resistance genes including TEM (30).


Molecular Epi *P. mirabilis* (PCR): 7 clinical isolates of *P. mirabilis* screened for β-lactamase resistance genes including TEM (3).

3.21. vanA

VanA confers resistance to the glycopeptide antibiotics vancomycin and teicoplanin in Gram-positive bacteria by modification of glycopeptide target. vanA detected in *E. faecalis* [136, 137]. VanA assay detects vanA gene.


Molecular Epi *E. faecalis* (DNA hybridization): 8 patients screened over 6 months for VRE (vancomycin resistant enterococcus) with 21 VRE isolates identified as *E. faecalis*.


Isolate Study *E. faecalis*: clinical isolate of *E. faecalis* carried vanA by whole genome sequencing.

3.22. VIM (VIM-1, VIM-2, VIM-5, VIM-13)

VIM is a large family of plasmid–encoded metallo-β-lactamases, which hydrolyze imipenem, meropenem, aztreonam, ceftazidime, piperacillin, tazobactam, cefepime, ciprofloxacin, tobramycin, amikacin, and gentamicin, and aztreonam at varying levels, although VIM-2, VIM-7, and VIM-13 poorly hydrolyze aztreonam. VIM activity blocked by β-lactamase inhibitors [138-140]. VIM reported in clinical strains of *E. coli* [141-143], *K. pneumoniae* [144-145], *P. aeruginosa* [109, 114, 138, 139, 147] and *P. mirabilis* [148]. VIM assay detects several gene subtypes across gene families VIM-1, VIM-2, VIM-5 and VIM-13.

Ref Error! Reference source not found.. Molecular Epi *P. aeruginosa* (WGS): VIM-2 (11), VIM-5 (2).

Ref 114. Molecular Epi *P. aeruginosa* (PCR): VIM-2 (1), VIM-4 (6).


Isolate Study *P. aeruginosa*: First characterization of VIM-7 in *P. aeruginosa*. 

**Isolate Study P. aeruginosa**: First characterization of VIM-13 in *P. aeruginosa*.


**Review Article**: metallo-β-lactamases reviewed including substrate specificity.


**Isolate Study E. coli**: Clinical isolate of *E. coli* possessing both GES-7 and VIM-2.


**Isolate Study E. coli**: First report of VIM-12.


**Isolate Study E. coli**: Clinical isolate of *E. coli* positive for VIM-1.


**Molecular Epi K. pneumoniae (PCR)**: 139 clinical isolates of *K. pneumoniae* screened for β-lactamase resistance genes including VIM-4 (1).


**Isolate Study K. pneumoniae**: First characterization of VIM-39 in *K. pneumoniae*.


**Molecular Epi K. pneumoniae (PCR)**: 50 clinical isolates of imipenem-resistant *K. pneumoniae* screened for VIM-1 (15).


**Isolate Study P. aeruginosa**: First report of VIM-2 in *P. aeruginosa*.
3.23. VEB

VEB is a family of plasmid- and integron-encoded β-lactamase. VEB confers resistance to ceftazidime, cefotaxime, aztreonam, and quinolones, plus penicillin to a lesser extent, but no resistance to moxalactam, imipenem, or cefoxitin. VEB activity blocked by β-lactamase inhibitors clavulanate, sulbactam, and tazobactam [149, 150]. VEB reported in clinical strains of *E. coli* [150], *P. aeruginosa* [151], *K. pneumoniae* [152] and *P. mirabilis* [153]. VEB assay detects several gene subtypes of veb.


**Review Article**: Less prevalent β-lactamases reviewed including substrate specificity.


**Isolate Study E. coli**: First report of VEB-1 in clinical isolate of *E. coli*.


**Isolate Study P. aeruginosa**: VEB-1 reported in a clinical isolate of *P. aeruginosa*.


**Molecular Epi K. pneumoniae (PCR)**: 150 clinical isolate of *K. pneumoniae* screened for β-lactam resistance genes including VEB-1 (14).


**Isolate Study P. mirabilis**: Clinical isolate of *P. mirabilis* characterized for antibiotic resistance genes and demonstrated to posses VEB-1.
4. OpGen Headquarters Location

<table>
<thead>
<tr>
<th>Corporate Headquarters</th>
</tr>
</thead>
<tbody>
<tr>
<td>OpGen, Inc.</td>
</tr>
<tr>
<td>9717 KeyWest Avenue Suite 100</td>
</tr>
<tr>
<td>Rockville, MD 20850</td>
</tr>
<tr>
<td>USA</td>
</tr>
<tr>
<td><strong>Telephone:</strong> +1 301 869 9683</td>
</tr>
<tr>
<td><strong>Fax:</strong> +1 301 869 9684</td>
</tr>
<tr>
<td><a href="http://www.opgen.com">www.opgen.com</a></td>
</tr>
</tbody>
</table>

5. Technical Assistance

<table>
<thead>
<tr>
<th>Region</th>
<th>Telephone</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>+1 301 869 9683</td>
<td><a href="mailto:technicalsupport@opgen.com">technicalsupport@opgen.com</a></td>
</tr>
</tbody>
</table>

OpGen, Acuitas and associated logos are registered trademarks of OpGen, Inc. All other trademarks that appear in this package insert are the property of their respective owners.

©2021 OpGen, Inc. All rights reserved.