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Acuitas® AMR Gene Panel

Electronic User Guide Literature Summary

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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.
5. **Review Article**: Literature review of antibiotics resistance gene studies in bacterial isolates.

Note Literature references indicated by square parentheses [ ].

Note 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.

1. Le Goffic, F, et al, *3-N enzymatic acetylation of gentamicin, tobramycin, and kanamycin by Escherichia coli carrying an R factor.* Antimicrob Agents Chemother, 1974. **6**(6): p. 680-4.

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.

2. Stoesser, N., et al., *Predicting antimicrobial susceptibilities for Escherichia coli and Klebsiella pneumoniae isolates using whole genomic sequence data.* J Antimicrob Chemother, 2013. 68(10): p. 2234-44.

   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)-IId (3) and aac(3)-IIe (19).

3. Xiao, Y. and Y. Hu, *The major aminoglycoside-modifying enzyme AAC(3)-II found in Escherichia coli determines a significant disparity in its resistance to gentamicin and amikacin in China.* Microb Drug Resist, 2012. **18**(1): p. 42-6.

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

4. Cirit OS, et al. Aminoglycoside resistance determinants in multi-resistant *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates from Turkish and Syrian patients. Acta Microbiol Immunol Hung. 2019 Sep 1;66(3):327-335.

   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).

5. Meradji S, et al. Epidemiology of carbapenem non-susceptible *Pseudomonas aeruginosa* isolates in Eastern Algeria. Antimicrob Resist Infect Control. 2015 Jun 12;4:27.

   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).

6. Fernandez-Martinez, M., et al., *Molecular identification of aminoglycoside-modifying enzymes in clinical isolates of Escherichia coli resistant to amoxicillin/clavulanate isolated in Spain.* Int J Antimicrob Agents, 2015. 46(2): p. 157-63.

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

7. Huang ZM, et al. Analysis on 168 rRNA methylase genes and aminoglycoside modifying enzymes genes in *Enterobacter cloacae* in China]. Zhonghua Liu Xing Bing Xue Za Zhi. 2008 Apr;29(4):369-73.

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

8. Miró E, et al. Characterization of aminoglycoside-modifying enzymes in *Enterobacteriaceae* clinical strains and characterization of the plasmids implicated in their diffusion. Microb Drug Resist. 2013 Apr;19(2):94-9.

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.

See Reference 8: **AST Study *E. coli, K. pneumoniae* (PCR)**: aac(3)-IVa: *K. pneumoniae* (22), *E. coli* (1).

9. Bräu B and Piepersberg W. Purification and characterization of a plasmid-encoded aminoglycoside-(3)-N-acetyltransferase IV from *Escherichia coli*. FEBS Lett. 1985 Jun 3;185(1):43-6. PubMed PMID: 3888671.3888671.

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

10. Johnson, A.P., et al., *Gentamicin resistance in clinical isolates of Escherichia coli encoded by genes of veterinary origin.* J Med Microbiol, 1994. **40**(3): p. 221-6.

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

11. Johnson AP, et al. Urinary isolates of apramycin-resistant *Escherichia coli* and *Klebsiella pneumoniae* from Dublin. Epidemiol Infect. 1995 Feb;114(1):105-12.

    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).

12. Almaghrabi, R., et al., *Carbapenem-resistant Klebsiella pneumoniae strains exhibit diversity in aminoglycoside-modifying enzymes, which exert differing effects on plazomicin and other agents.* Antimicrob Agents Chemother, 2014. **58**(8): p. 4443-51.

    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).

13. Boudjemaa H, et al. Molecular drivers of emerging multidrug resistance in *Proteus mirabilis* clinical isolates from Algeria. J Glob Antimicrob Resist. 2019 Feb 20. pii: S2213-7165(19)30037-2.

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

14. Holbrook SYL and Garneau-Tsodikova S. Evaluation of Aminoglycoside and Carbapenem Resistance in a Collection of Drug-Resistant *Pseudomonas aeruginosa* Clinical Isolates. Microb Drug Resist. 2018 Sep;24(7):1020-1030.

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

15. Momtaz H, et al. Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing *Escherichia coli* isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathog. 2013 Dec 11;5(1):39.

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')-Ib/Ib-cr

AAC(6')-Ib is an aminoglycoside 6'-N-acetyltransferase. aac(6')-Ib-cr is a variant of aac(6')-Ib 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')-Ib-cr, Trp102Arg and Asp179Tyr, are together necessary to acetylate ciprofloxacin [16]. aac(6')-Ib and aac(6')-Ib-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')-Ib/Ib-cr assay detects gene subtypes aac(6')-Ib, aac(6')Ib-cr, aacA4, aacA4-8, ant(3'')-Ih-aac(6')-IId and aac(3)-Ib-aac(6')-Ib.

See Reference 8: **AST Study *E. coli, K. pneumoniae, P. mirabilis, Enterobacteriaceae* (PCR)**: aac(6')-Ib renders resistance in *E. coli* (102), *K. pneumoniae* (154), *P. mirabilis* (3) and other *Enterobacteriaceae K. oxytoca* (6), *E. cloacae* (4).

See Reference 12: **AST Study *K. pneumoniae* (PCR)**: aac(6')-1b (49).

16. Robicsek, A., et al., *Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase.* Nat Med, 2006. **12**(1): p. 83-8.

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

17. Pitout, J.D., et al., *Surveillance for plasmid-mediated quinolone resistance determinants in Enterobacteriaceae within the Calgary Health Region, Canada: the emergence of aac(6')-Ib-cr.* J Antimicrob Chemother, 2008. **61**(5): p. 999-1002.

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

18. Shin, S.Y., et al., *Characteristics of aac(6')-Ib-cr gene in extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolated from Chungnam area.* Korean J Lab Med, 2009. **29**(6): p. 541-50.

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.

19. Holbrook, S.Y.L. and S. Garneau-Tsodikova, *Evaluation of Aminoglycoside and Carbapenem Resistance in a Collection of Drug-Resistant Pseudomonas aeruginosa Clinical Isolates.* Microb Drug Resist, 2017.

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

20. Wieczorek, P., et al., *The aac(6')Ib gene in Proteus mirabilis strains resistant to aminoglycosides.* Folia Histochem Cytobiol, 2008. **46**(4): p. 531-3.

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

21. Leulmi Z, et al. First report of blaOXA-24 carbapenemase-encoding gene, armA Methyltransferase and aac(6)-Ib-cr producing multidrug-resistant clinical isolates of *Proteus mirabilis* in Algeria. J Glob Antimicrob Resist. 2018 Sep 11. pii: S2213-7165(18)30166-8.

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-transferase (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.

See Reference 21: **Molecular Epi *P. mirabilis*, *Enterobacteriaceae* (PCR):** aadA2: *P. mirabilis* (4) and other *Enterobacteriaceae* *P. vulgaris* (1) and *M. morganii* (1).

22. Shahcheraghi F,et al. Identification and characterization of class 1 integrons among atypical enteropathogenic *Escherichia coli* isolated from children under 5 years of age. Iran J Microbiol. 2014 Jun;6(3):156-62.

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

23. Fling, M.E., J. et al, *Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase.* Nucleic Acids Res, 1985. **13**(19): p. 7095-106.

Mechanistic Study: Nucleotide sequence of aadA deduced.

24. Peters, E.D., et al., *Novel gene cassettes and integrons.* Antimicrob Agents Chemother, 2001. **45**(10): p. 2961-4.

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).

25. Kazama, H., et al., *A new gene, aadA2b, encoding an aminoglycoside adenylyltransferase, AAD(3")(9), isolated from integron InC in Pseudomonas aeruginosa.* Microbios, 1996. **86**(347): p. 77-83.

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

26. Chen DQ, et al. Integron mediated bacterial resistance and virulence on clinical pathogens. Microb Pathog. 2018 Jan;114:453-457.

Molecular Epi *K. pneumoniae, P. aeruginosa, E. faecalis, Enterobacteriaceae* (LAMP): antibiotic resistance genes on various integrons characterized in 22 clinical isolates including aadA2: *K. pneumoniae* (2), *P. aeruginosa* (2), *E. faecalis* (3), and other *Enterobacteriaceae Enterobacter* sp. (3).

27. Chen, C.M., et al., *Genetic characteristic of class 1 integrons in proteus mirabilis isolates from urine samples.* Biomedicine (Taipei), 2017. **7**(2): p. 9.

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

28. Clark, N.C., et al., *Detection of a streptomycin/spectinomycin adenylyltransferase gene (aadA) in Enterococcus faecalis.* Antimicrob Agents Chemother, 1999. **43**(1): p. 157-60.

    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).

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

See Reference 8: **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).

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

See Reference 21: **Molecular Epi *P. mirabilis, Enterobacteriaceae* (PCR):** ant(2")-Ia: *P. mirabilis* (1) and other *Enterobacteriaceae P. vulgaris* (2) and *M. morganii* (1).

29. Mozes, J., et al., *A potential role of aminoglycoside resistance in endemic occurrence of Pseudomonas aeruginosa strains in lower airways of mechanically ventilated patients.* Diagn Microbiol Infect Dis, 2014. **78**(1): p. 79-84.

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

30. Costello SE, et al. Aminoglycoside-modifying enzyme and 16S ribosomal RNA methyltransferase genes among a global collection of Gram-negative isolates. J Glob Antimicrob Resist. 2019 Mar;16:278-285.

    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.

31. Rao, R.N., et al., *Genetic and enzymatic basis of hygromycin B resistance in Escherichia coli.* Antimicrob Agents Chemother, 1983. **24**(5): p. 689-95.

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

32. Fu L, et al. Co-carrying of KPC-2, NDM-5, CTX-M-3 and CTX-M-65 in three plasmids with serotype O89: H10 *Escherichia coli* strain belonging to the ST2 clone in China. Microb Pathog. 2019 Mar;128:1-6.

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

33. Yu CY, et al. Genome Sequences of Two Multidrug-Resistant Proteus mirabilis Strains Harboring CTX-M-65 Isolated from Malaysia. Genome Announc. 2016 Nov 17;4(6). pii: e01301-16.

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).

34. Ruan Z, et al. Emergence of a ST2570 *Klebsiella pneumoniae* isolate carrying mcr-1 and bla(CTX-M-14) recovered from a bloodstream infection in China. Clin Microbiol Infect. 2019 Jul;25(7):916-918.

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.

See Reference 21: **Molecular Epi *P. mirabilis Enterobacteriaceae* (PCR):** armA: *P. mirabilis* (1) and other *Enterobacteriaceae* *P. vulgaris* (1).

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

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

35. Galimand, M., et al, *Plasmid-mediated high-level resistance to aminoglycosides in Enterobacteriaceae due to 16S rRNA methylation.* Antimicrob Agents Chemother, 2003. **47**(8): p. 2565-71.

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

36. Shrestha B, et al. Emergence of Various NDM-Type-Metallo-β-Lactamase-Producing *Escherichia coli* Clinical Isolates in Nepal. Antimicrob Agents Chemother. 2017 Nov 22;61(12). pii: e01425-17.

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).

37. Yan, J.J., et al., *Plasmid-mediated 16S rRNA methylases conferring high-level aminoglycoside resistance in Escherichia coli and Klebsiella pneumoniae isolates from two Taiwanese hospitals.* J Antimicrob Chemother, 2004. **54**(6): p. 1007-12.

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).

38. Zacharczuk, K., et al., *Plasmid-borne 16S rRNA methylase ArmA in aminoglycoside-resistant Klebsiella pneumoniae in Poland.* J Med Microbiol, 2011. **60**(Pt 9): p. 1306-11.

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

39. Mohanam L, et al. Emergence of rmtC and rmtF 16S rRNA methyltransferase in clinical isolates of *Pseudomonas aeruginosa*. Indian J Med Microbiol. 2017 Apr-Jun;35(2):282-285.

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

40. Wu, J.J., et al., *Prevalence of extended-spectrum beta-lactamases in Proteus mirabilis in a Taiwanese university hospital, 1999 to 2005: identification of a novel CTX-M enzyme (CTX-M-66).* Diagn Microbiol Infect Dis, 2008. **60**(2): p. 169-75.

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

41. Lee, H., et al., *Dissemination of 16S rRNA methylase-mediated highly amikacin-resistant isolates of Klebsiella pneumoniae and Acinetobacter baumannii in Korea.* Diagn Microbiol Infect Dis, 2006. **56**(3): p. 305-12.

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).

42. Rahman, M., et al., *RmtC and RmtF 16S rRNA Methyltransferase in NDM-1-Producing Pseudomonas aeruginosa.* Emerg Infect Dis, 2015. **21**(11): p. 2059-62.

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).

43. Zhou, Y., et al., *Distribution of 16S rRNA methylases among different species of Gram-negative bacilli with high-level resistance to aminoglycosides.* Eur J Clin Microbiol Infect Dis, 2010. **29**(11): p. 1349-53.

Molecular Epi *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, Enterobacteriaceae* (PCR): 217 amikacin/gentamicin-resistant Gram-negative clinical isolates screened for aminoglyside 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 cephamycins (cephamycinase, 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.

44. Chen, Y.T., et al., *KPC-2-encoding plasmids from Escherichia coli and Klebsiella pneumoniae in Taiwan.* J Antimicrob Chemother, 2014. **69**(3): p. 628-31.

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

45. Matsumura, Y., et al., *High prevalence of carbapenem resistance among plasmid-mediated AmpC beta-lactamase-producing Klebsiella pneumoniae during outbreaks in liver transplantation units.* Int J Antimicrob Agents, 2015. **45**(1): p. 33-40.

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

46. Seiffert, S.N., et al., *Emergence of Klebsiella pneumoniae co-producing NDM-1, OXA-48, CTX-M-15, CMY-16, QnrA and ArmA in Switzerland.* Int J Antimicrob Agents, 2014. **44**(3): p. 260-2.

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

47. Harris, P.N.A., et al., *Whole genome analysis of cephalosporin-resistant Escherichia coli from bloodstream infections in Australia, New Zealand and Singapore: high prevalence of CMY-2 producers and ST131 carrying blaCTX-M-15 and blaCTX-M-27.* 2018 Mar 1;73(3):634-642.

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

48. Manageiro, V., et al., *Genetic Background and Expression of the New qepA4 Gene Variant Recovered in Clinical TEM-1- and CMY-2-Producing Escherichia coli.* Front Microbiol, 2017. **8**: p. 1899.

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

49. D'Andrea, M.M., et al., *CMY-16, a novel acquired AmpC-type beta-lactamase of the CMY/LAT lineage in multifocal monophyletic isolates of Proteus mirabilis from northern Italy.* Antimicrob Agents Chemother, 2006. **50**(2): p. 618-24.

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

50. Mata, C., et al., *Prevalence of SXT/R391-like integrative and conjugative elements carrying blaCMY-2 in Proteus mirabilis.* J Antimicrob Chemother, 2011. **66**(10): p. 2266-70.

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

51. Upadhyay, S., et al., *Co-existence of Pseudomonas-derived cephalosporinase among plasmid encoded CMY-2 harbouring isolates of Pseudomonas aeruginosa in north India.* Indian J Med Microbiol, 2013. **31**(3): p. 257-60.

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

52. Yan, J.J., et al., *Dissemination of CTX-M-3 and CMY-2 beta-lactamases among clinical isolates of Escherichia coli in southern Taiwan.* J Clin Microbiol, 2000. **38**(12): p. 4320-5.

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

53. Cha, M.K., et al., *High Prevalence of CTX-M-15-Type Extended-Spectrum beta-Lactamase Among AmpC beta-Lactamase-Producing Klebsiella pneumoniae Isolates Causing Bacteremia in Korea.* Microb Drug Resist. 2018 Sep;24(7):1002-1005.

    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.

54. Tzouvelekis, L.S., et al., *CTX-M-type beta-lactamases: an emerging group of extended-spectrum enzymes.* Int J Antimicrob Agents, 2000. **14**(2): p. 137-42.

    Review Article: CTX-M beta lactamases were reviewed including moleular 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*.

55. Pavez M, et al . High prevalence of CTX-M-1 group in ESBL-producing *Enterobacteriaceae* infection in intensive care units in southern Chile. Braz J Infect Dis. 2019 Mar - Apr;23(2):102-110.

    Molecular Epi *E. coli*, *K. pneumoniae*, *P. mirabilis* and *Enterobacteriaceae* (PCR): 137 ESBL-producing bacteria screened for beta-lactamase genes including 115 clinical isolates of *K. pneumoniae*: CTX-M-1 variants (98), CTX-M-2 variants (25), CTX-M-9 variants (1), 18 clinical isolates of *E. coli*: CTX-M1 variants (15), CTX-M-2 variants (2), CTX-M-9 variants (12), 3 clinical iolates of *P. mirabilis*: CTX-M-1 variants (3), CTX-M-2 variants (3), CTX-M-9 variants (2), and 3 clinical isolates of *Enterobacteriaceae E. cloacae*: CTX-M-2 variants (1).

56. Shams E, et al. Prevalence of Plasmid-Mediated Quinolone Resistance Genes among Extended-Spectrum β -Lactamase-Producing *Klebsiella pneumoniae* Human Isolates in Iran. J Pathog. 2015;2015:434391.

    Molecular Epi *K. pneumoniae* (PCR): 70 *K. pneumoniae* clinical isolates screened for CTX-M genes: CTX-M-A (42), CTX-M-2 (30), CTX-M-9 (24).

57. Bonnet, R., et al., *A novel CTX-M beta-lactamase (CTX-M-8) in cefotaxime-resistant Enterobacteriaceae isolated in Brazil.* Antimicrob Agents Chemother, 2000. **44**(7): p. 1936-42.

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

58. Picao, R.C., et al., *Further identification of CTX-M-2 extended-spectrum beta-lactamase in Pseudomonas aeruginosa.* Antimicrob Agents Chemother, 2009. **53**(5): p. 2225-6.

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, dfr17 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.

See Reference 26: **Molecular Epi *K. pneumoniae, P. aeruginosa, E. faecalis, Enterobacteriaceae* (LAMP)**: dfrA17: *E. faecalis* (1), *K. pneumoniae* (1), *P. aeruginosa* (1), other *Enterobacteriaceae Enterobacter sp.* (1).

59. Yu, H.S., et al., *Prevalence of dfr genes associated with integrons and dissemination of dfrA17 among urinary isolates of Escherichia coli in Korea.* J Antimicrob Chemother, 2004. **53**(3): p. 445-50.

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

60. Lee, J.C., et al., *The prevalence of trimethoprim-resistance-conferring dihydrofolate reductase genes in urinary isolates of Escherichia coli in Korea.* J Antimicrob Chemother, 2001. **47**(5): p. 599-604.

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

61. Brolund, A., et al., *Molecular characterisation of trimethoprim resistance in Escherichia coli and Klebsiella pneumoniae during a two year intervention on trimethoprim use.* PLoS One, 2010. **5**(2): p. e9233.

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).

62. Salimizand, H., et al., *Molecular characterization of class 1 integrons and gene cassettes in multidrug resistant (MDR) Klebsiella spp. isolated from hospitalized and outpatients in Iran, 2009.* Iran J Microbiol, 2013. **5**(1): p. 48-55.

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

63. Alabi OS, et al. Molecular screening of antibiotic-resistant determinants among multidrug-resistant clinical isolates of *Proteus mirabilis* from SouthWest Nigeria. Afr Health Sci. 2017 Jun;17(2):356-365.

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 cephamycins. DHA function demonstrated by transferring resistance to oxyimino-cephalosporins (cefotaxime and ceftazidime) and cephamycins (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).

See Reference 45: **Molecular Epi *K. pneumoniae* (PCR)**: DHA-1 (20).

See Reference 47: **Isolate Study *E. coli* (WGS):** DHA-1 (1).

See Reference 56: **Molecular Epi *K. pneumoniae* (PCR)**: DHA-1 (52).

64. Giakkoupi, P., et al., *Transferable DHA-1 cephalosporinase in Escherichia coli.* Int J Antimicrob Agents, 2006. **27**(1): p. 77-80.

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

65. Kis, Z., et al., *Countrywide dissemination of a DHA-1-type plasmid-mediated AmpC beta-lactamase-producing Klebsiella pneumoniae ST11 international high-risk clone in Hungary, 2009-2013.* J Med Microbiol, 2016. **65**(9): p. 1020-7.

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

66. Fortineau, N., L. Poirel, and P. Nordmann, *Plasmid-mediated and inducible cephalosporinase DHA-2 from Klebsiella pneumoniae.* J Antimicrob Chemother, 2001. **47**(2): p. 207-10.

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

67. Wu, L.T., et al., *Identification of a novel cephalosporinase (DHA-3) in Klebsiella pneumoniae isolated in Taiwan.* Clin Microbiol Infect, 2005. **11**(11): p. 893-7.

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

68. Bidet, P., et al., *First description of DHA-1 ampCbeta-lactamase in Proteus mirabilis.* Clin Microbiol Infect, 2005. **11**(7): p. 591-2.

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.

69. Conrad, S., et al., *gyrA mutations in high-level fluoroquinolone-resistant clinical isolates of Escherichia coli.* J Antimicrob Chemother, 1996. **38**(3): p. 443-55.

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

70. Fu, Y., et al., *Specific patterns of gyrA mutations determine the resistance difference to ciprofloxacin and levofloxacin in Klebsiella pneumoniae and Escherichia coli.* BMC Infect Dis, 2013. **13**: p. 8.

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

71. Hocquet, D., et al., *Genetic and phenotypic variations of a resistant Pseudomonas aeruginosa epidemic clone.* Antimicrob Agents Chemother, 2003. **47**(6): p. 1887-94.

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.

72. Koh, T.H., et al., *Carbapenem-hydrolysing IMP-1 beta-lactamase in Klebsiella pneumoniae from Singapore.* Lancet, 1999. **353**(9170): p. 2162.

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

73. Yan, J.J., W.C. Ko, and J.J. Wu, *Identification of a plasmid encoding SHV-12, TEM-1, and a variant of IMP-2 metallo-beta-lactamase, IMP-8, from a clinical isolate of Klebsiella pneumoniae.* Antimicrob Agents Chemother, 2001. **45**(8): p. 2368-71.

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

74. Peymani, A., et al., *Dissemination of Pseudomonas aeruginosa producing bla IMP-1 and bla VIM-1 in Qazvin and Alborz educational hospitals, Iran.* Iran J Microbiol, 2015. **7**(6): p. 302-9.

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

75. Tada, T., et al., *Multidrug-Resistant Sequence Type 235 Pseudomonas aeruginosa Clinical Isolates Producing IMP-26 with Increased Carbapenem-Hydrolyzing Activities in Vietnam.* Antimicrob Agents Chemother, 2016. **60**(11): p. 6853-6858.

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).

76. Gilarranz, R., et al., *First detection in Europe of the metallo-beta-lactamase IMP-15 in clinical strains of Pseudomonas putida and Pseudomonas aeruginosa.* Clin Microbiol Infect, 2013. **19**(9): p. E424-7.

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

77. Dixon, N., et al., *IMP-27, a Unique Metallo-beta-Lactamase Identified in Geographically Distinct Isolates of Proteus mirabilis.* Antimicrob Agents Chemother, 2016. **60**(10): p. 6418-21.

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

78. Potter, R.F., et al., *blaIMP-27 on transferable plasmids in Proteus mirabilis and Providencia rettgeri.* Clin Microbiol Infect, 2018. Sep;**24**(9):1019.e5-1019.e8.

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

79. Komatsu Y, et al. Molecular epidemiology and clinical features of extended-spectrum beta-lactamase- or carbapenemase-producing Escherichia coli bacteremia in Japan. PLoS One. 2018 Aug 29;13(8):e0202276.

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.

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

80. Yigit, H., et al., *Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae.* Antimicrob Agents Chemother, 2001. **45**(4): p. 1151-61.

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

81. Stoesser, N., et al., *Genomic epidemiology of global Klebsiella pneumoniae carbapenemase (KPC)-producing Escherichia coli.* Sci Rep, 2017. **7**(1): p. 5917.

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

82. Hagemann, J.B., et al., *KPC-2 carbapenemase-producing Pseudomonas aeruginosa reaching Germany.* J Antimicrob Chemother, 2018. Jul 1;**73**(7):1812-1814.

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

83. Tibbetts, R., et al., *Detection of KPC-2 in a clinical isolate of Proteus mirabilis and first reported description of carbapenemase resistance caused by a KPC beta-lactamase in P. mirabilis.* J Clin Microbiol, 2008. **46**(9): p. 3080-3.

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.

84. Mendes, A.C., et al., *mcr-1 in Carbapenemase-Producing Klebsiella pneumoniae with Hospitalized Patients, Portugal, 2016-2017.* Emerg Infect Dis, 2018. **24**(4): p. 762-766.

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

85. Zhong YM, et al., Epidemiology and molecular characterization of mcr-1 in *Escherichia coli* recovered from patients with bloodstream infections in Changsha, central China. Infect Drug Resist. 2019 Jul 12;12:2069-2076.

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

86. Cannatelli A, et al. Synergistic Activity of Colistin in Combination With Resveratrol Against Colistin-Resistant Gram-Negative Pathogens. Front Microbiol. 2018 Aug 7;9:1808.

    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).

87. Lai CC, et al. Susceptibility rates of clinically important bacteria collected from intensive care units against colistin, carbapenems, and other comparative agents: results from Surveillance of Multicenter Antimicrobial Resistance in Taiwan(SMART). Infect Drug Resist. 2019 Mar 14;12:627-640.

    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.

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

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

88. Yong, D., et al., *Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India.* Antimicrob Agents Chemother, 2009. **53**(12): p. 5046-54.

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

89. Deshpande, P., et al., *New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae : treatment options with carbapenems compromised.* J Assoc Physicians India, 2010. **58**: p. 147-9.

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).

90. Mulvey, M.R., et al., *New Delhi metallo-beta-lactamase in Klebsiella pneumoniae and Escherichia coli, Canada.* Emerg Infect Dis, 2011. **17**(1): p. 103-6.

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

91. Jovcic, B., et al., *Emergence of NDM-1 metallo-beta-lactamase in Pseudomonas aeruginosa clinical isolates from Serbia.* Antimicrob Agents Chemother, 2011. **55**(8): p. 3929-31.

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

92. Qin, S., et al., *Emergence of Extensively Drug-Resistant Proteus mirabilis Harboring a Conjugative NDM-1 Plasmid and a Novel Salmonella Genomic Island 1 Variant, SGI1-Z.* Antimicrob Agents Chemother, 2015. **59**(10): p. 6601-4.

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

93. Valentin, T., et al., *Proteus mirabilis harboring carbapenemase NDM-5 and ESBL VEB-6 detected in Austria.* Diagn Microbiol Infect Dis, 2018.

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 oxacillinases 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.

94. Torres, E., et al., *Reduced Susceptibility to Cefepime in Clinical Isolates of Enterobacteriaceae Producing OXA-1 Beta-Lactamase.* Microb Drug Resist, 2016. **22**(2): p. 141-6.

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

95. Bitar, I., et al., *First report of an Escherichia coli from Lebanon carrying an OXA-181 carbapenemase resistance determinant.* J Glob Antimicrob Resist, 2018. **12**: p. 113-114.

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

96. Hoyos-Mallecot, Y., et al., *OXA-244-Producing Escherichia coli Isolates, a Challenge for Clinical Microbiology Laboratories.* Antimicrob Agents Chemother, 2017. Aug 24;61(9). pii: e00818-17.

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

97. Jao, Y.T., et al., *First report of OXA-48 carbapenemase-producing Escherichia coli in Taiwan.* J Microbiol Immunol Infect, 2017. **50**(3): p. 403-404.

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

98. Bonnin, R.A., et al., *Molecular characterization of OXA-198 carbapenemase producing Pseudomonas aeruginosa clinical isolates.* Antimicrob Agents Chemother, 2018. 25;62(6). pii: e02496-17.

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

99. Aktas, Z., et al., *Carbapenem-hydrolyzing oxacillinase, OXA-48, persists in Klebsiella pneumoniae in Istanbul, Turkey.* Chemotherapy, 2008. **54**(2): p. 101-6.

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

100. Cubero, M., et al., *Clonal spread of Klebsiella pneumoniae producing OXA-1 betalactamase in a Spanish hospital.* Int Microbiol, 2013. **16**(4): p. 227-33.

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

101. Sugumar, M., et al., *Detection of OXA-1 beta-lactamase gene of Klebsiella pneumoniae from blood stream infections (BSI) by conventional PCR and in-silico analysis to understand the mechanism of OXA mediated resistance.* PLoS One, 2014. **9**(3): p. e91800.

    Molecular Epi *K. pneumoniae* (PCR): 59 clinical isolates of *K. pneumoniae* screened for OXA-48 (12).

102. Bojorquez D, et al. 1998. Cell Mol Biol (Noisy-le-grand) 44(3): 483-491. Characterization of OXA-9, a beta-lactamase encoded by the multiresistance transposon Tn1331. (PMID 9620445).

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

103. Chen, L., et al., *First report of an OXA-48-producing multidrug-resistant Proteus mirabilis strain from Gaza, Palestine.* Antimicrob Agents Chemother, 2015. **59**(7): p. 4305-7.

    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 cephamycins. *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.

See Reference 55: **Molecular Epi *K. pneumoniae* (PCR):** PER-1: *K. pneumoniae* (1).

104. Nordmann, P., et al., *Characterization of a novel extended-spectrum beta-lactamase from Pseudomonas aeruginosa.* Antimicrob Agents Chemother, 1993. **37**(5): p. 962-9.

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

105. Erac, B., et al., *Prevalence of blaPER-1 and integrons in ceftazidime-resistant Gram-negative bacteria at a university hospital in Turkey.* Jpn J Infect Dis, 2013. **66**(2): p. 146-8.

Molecular Epi *E. coli*, *K. pneumoniae*, *P. aeruginosa* (PCR): Ceftazidime-resistant clinical isolates of Gram-negative bacilli screened for PER-1: *E. coli* (11 of 16). *K. pneumoniae* (5 of 10), *P. aeruginosa* (17 of 73).

106. Quinteros, M., et al., *Extended-spectrum beta-lactamases in Enterobacteriaceae in Buenos Aires, Argentina, public hospitals.* Antimicrob Agents Chemother, 2003. **47**(9): p. 2864-7.

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).

107. Maurya AP, et al. Emergence of integron borne PER-1 mediated extended spectrum cephalosporin resistance among nosocomial isolates of Gram-negative bacilli. Indian J Med Res. 2015 Jun;141(6):816-22.

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.

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

See Reference 37: **Molecular Epi *E. coli K. pneumoniae* (PCR)**: rmtB in *E. coli* (2) and *K. pneumoniae* (5).

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

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

See Reference 41: **Molecular Epi *K. pneumoniae* (PCR):** rmtB (1).

See Reference 42: **Molecular Epi *P. aeruginosa* (PCR)**: rmtF (10).

See Reference 43: **Molecular Epi *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* (PCR):** rmtB: *E. coli* (9), *K. pneumoniae* (36), *P. mirabilis* (7), *P. aeruginosa* (5).

108. Galani, et al. Prevalence of 16S rRNA methylase genes in *Enterobacteriaceae* isolates from a Greek university hospital. Clin Microbiol Infect. 2012 Mar;18(3):E52-4.

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

109. Tada T, et al. Molecular Characterization of Multidrug-Resistant *Pseudomonas aeruginosa* Isolates in Hospitals in Myanmar. Antimicrob Agents Chemother. 2019 63(5). pii: e02397-18.

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

110. Tada T, et al. Dissemination of Carbapenem-resistant *Klebsiella pneumoniae* clinical isolates with various combinations of Carbapenemases (KPC-2, NDM-1, NDM-4, and OXA-48) and 16S rRNA Methylases (RmtB and RmtC) in Vietnam. BMC Infect Dis. 2017 17(1):467.

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

111. Galimand, M., P. Courvalin, and T. Lambert, *RmtF, a new member of the aminoglycoside resistance 16S rRNA N7 G1405 methyltransferase family.* Antimicrob Agents Chemother, 2012. **56**(7): p. 3960-2.

    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*.

112. Hidalgo, L., et al., *Association of the novel aminoglycoside resistance determinant RmtF with NDM carbapenemase in Enterobacteriaceae isolated in India and the UK.* J Antimicrob Chemother, 2013. **68**(7): p. 1543-50.

    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).

113. Yan J, et al. Multidrug Resistance Mechanisms of Carbapenem Resistant *Klebsiella pneumoniae* Strains Isolated in Chongqing, China. Ann Lab Med. 2017 **37**(5):398-407.

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

114. Meradji S, et al. Epidemiology and virulence of VIM-4 metallo-beta-lactamase-producing *Pseudomonas aeruginosa* isolated from burn patients in eastern Algeria. Burns. 2016. **42**(4):906-18.

    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.

See Reference 2: **Molecular Epi *K. pneumoniae* (WGS)**: SHV variants *K. pneumoniae* (61): SHV-1 (28), SHV-11 (19), SHV-28 (4), SHV-33 (2), SHV-121 (2), SHV-27 (1), SHV-60 (1), SHV-135 (1).

See Reference 55: **Molecular Epi *E. coli, K. pneumoniae, P. mirabilis* (PCR):** SHV-like: *E. coli* (6), *K. pneumoniae* (103), *P. mirabilis* (2).

See Reference 110: **Molecular Epi *K. pneumoniae* (WGS):** SHV-1 (4), SHV-11 (1), SHV-12 (3), SHV-28 (18).

115. Chaves, J., et al., *SHV-1 beta-lactamase is mainly a chromosomally encoded species-specific enzyme in Klebsiella pneumoniae.* Antimicrob Agents Chemother, 2001. **45**(10): p. 2856-61.

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

116. Jones, C.H., et al., *Pyrosequencing using the single-nucleotide polymorphism protocol for rapid determination of TEM- and SHV-type extended-spectrum beta-lactamases in clinical isolates and identification of the novel beta-lactamase genes blaSHV-48, blaSHV-105, and blaTEM-155.* Antimicrob Agents Chemother, 2009. **53**(3): p. 977-86.

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

117. Gutmann, L., et al., *SHV-5, a novel SHV-type beta-lactamase that hydrolyzes broad-spectrum cephalosporins and monobactams.* Antimicrob Agents Chemother, 1989. **33**(6): p. 951-6.

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

118. Nicolas, M.H., et al., *Molecular characterization of the gene encoding SHV-3 beta-lactamase responsible for transferable cefotaxime resistance in clinical isolates of Klebsiella pneumoniae.* Antimicrob Agents Chemother, 1989. **33**(12): p. 2096-100.

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

119. Pourakbari B, et al. Molecular characteristics and antibiotic resistance profiles of Escherichia coli strains isolated from urinary tract infections in children admitted to children's referral hospital of Qom, Iran. Ann Ig. 2019 May-Jun;31(3):252-262.

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

120. Bahmani, N. and R. Ramazanzadeh, *Detection of SHV type Extended-Spectrum B-lactamase and Risk Factors in Pseudomonas aeruginosa Clinical Isolates.* Pak J Med Sci, 2013. **29**(3): p. 788-92.

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

121. Jones, CH, et al., Pyrosequencing using the single-nucleotide polymorphism protocol for rapid determination of TEM- and SHV-type extended-spectrum beta-lactamases in clinical isolates and identification of the novel beta-lactamase genes blaSHV-48, blaSHV-105, and blaTEM-155. Antimicrob Agents Chemother. 2009 53(3):977-86.

    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.

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

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

122. Enne, V.I., et al., *Persistence of sulphonamide resistance in Escherichia coli in the UK despite national prescribing restriction.* Lancet, 2001. **357**(9265): p. 1325-8.

Moleular 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.

123. Radstrom, P. and G. Swedberg, *RSF1010 and a conjugative plasmid contain sulII, one of two known genes for plasmid-borne sulfonamide resistance dihydropteroate synthase.* Antimicrob Agents Chemother, 1988. **32**(11): p. 1684-92.

Mechanistic Study: nucleotide sequence of sul2 reported.

124. Jeong, S., et al., *Extensively Drug-Resistant Escherichia coli Sequence Type 1642 Carrying an IncX3 Plasmid Containing the blaKPC-2 Gene Associated with Transposon Tn4401a.* Ann Lab Med, 2018. **38**(1): p. 17-22.

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

125. Wu, S., et al., *Prevalence and characterization of plasmids carrying sulfonamide resistance genes among Escherichia coli from pigs, pig carcasses and human.* Acta Vet Scand, 2010. **52**: p. 47.

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

126. Bissonnette, L. and P.H. Roy, *Characterization of In0 of Pseudomonas aeruginosa plasmid pVS1, an ancestor of integrons of multiresistance plasmids and transposons of gram-negative bacteria.* J Bacteriol, 1992. **174**(4): p. 1248-57.

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

127. Siebor E, et al. Genomic context of resistance genes within a French clinical MDR Proteus mirabilis: identification of the novel genomic resistance island GIPmi1. J Antimicrob Chemother. 2018 73(7):1808-1811.

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

128. Esposito EP, et al. A Novel IncA/C1 Group Conjugative Plasmid, Encoding VIM-1 Metallo-Beta-Lactamase, Mediates the Acquisition of Carbapenem Resistance in ST104 Klebsiella pneumoniae Isolates from Neonates in the Intensive Care Unit of V. Monaldi Hospital in Naples. Front Microbiol. 2017 Nov 3;8:2135.

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, 130, 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.

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

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

See Reference 55: **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).

See Reference 110: **Molecular Epi *K. pneumoniae* (WGS):** TEM-1 (25).

129. Arisawa, M. and R. Then, *Inactivation of TEM-1 beta-lactamase by 6-acetylmethylenepenicillanic acid.* Biochem J, 1983. **209**(3): p. 609-15.

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

130. Simpson, I.N., Pet al., *Principal beta-lactamases responsible for resistance to beta-lactam antibiotics in urinary tract infections.* Antimicrob Agents Chemother, 1980. **17**(6): p. 929-36.

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

131. Petit, A., et al., *Multiple substitutions at position 104 of beta-lactamase TEM-1: assessing the role of this residue in substrate specificity.* Biochem J, 1995. **305 (Pt 1)**: p. 33-40.

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

132. Venkatachalam, K.V., et al., *Characterization of TEM-1 beta-lactamase mutants from positions 238 to 241 with increased catalytic efficiency for ceftazidime.* J Biol Chem, 1994. **269**(38): p. 23444-50.

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

133. Hernandez, J.R., et al., *Nationwide study of Escherichia coli and Klebsiella pneumoniae producing extended-spectrum beta-lactamases in Spain.* Antimicrob Agents Chemother, 2005. **49**(5): p. 2122-5.

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).

134. Bokaeian, M., et al., *Frequency of PER, VEB, SHV, TEM and CTX-M Genes in Resistant Strains of Pseudomonas aeruginosa Producing Extended Spectrum beta-Lactamases.* Jundishapur J Microbiol, 2015. **8**(1): p. e13783.

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

135. Sardelic, S., et al., *Emergence of Proteus mirabilis isolates producing TEM-52 extended-spectrum beta-lactamases in Croatia.* Chemotherapy, 2010. **56**(3): p. 208-13.

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.

136. Biavasco, F., et al., *Genotypic characterization of a nosocomial outbreak of VanA Enterococcus faecalis.* Microb Drug Resist, 1996. **2**(2): p. 231-7.

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*.

137. Strateva T, et al. First detection and characterization of a VanA-type *Enterococcus faecalis* clinical isolate from Bulgaria. J Glob Antimicrob Resist. 2019 Aug 6. pii: S2213-7165(19)30196-1.

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, 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.

See Reference 109: **Molecular Epi *P. aeruginosa* (WGS):** VIM-2 (11), VIM-5 (2).

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

138. Toleman, M.A., et al., *blaVIM-7, an evolutionarily distinct metallo-beta-lactamase gene in a Pseudomonas aeruginosa isolate from the United States.* Antimicrob Agents Chemother, 2004. **48**(1): p. 329-32.

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

139. Juan, C., et al., *Characterization of the new metallo-beta-lactamase VIM-13 and its integron-borne gene from a Pseudomonas aeruginosa clinical isolate in Spain.* Antimicrob Agents Chemother, 2008. **52**(10): p. 3589-96.

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

140. Walsh, T., et al., *Metallo-ß-Lactamases: the Quiet before the Storm?* Clin Microbiol Rev., 2005. **18**(2): p. 306–325.

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

141. Galani, I., et al., *Molecular characterization of an Escherichia coli clinical isolate that produces both metallo-beta-lactamase VIM-2 and extended-spectrum beta-lactamase GES-7: identification of the In8 integron carrying the blaVIM-2 gene.* J Antimicrob Chemother, 2006. **58**(2): p. 432-3.

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

142. Ikonomidis, A., et al., *First occurrence of an Escherichia coli clinical isolate producing the VIM-1/VIM-2 hybrid metallo-beta-lactamase VIM-12.* Antimicrob Agents Chemother, 2007. **51**(8): p. 3038-9.

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

143. Miriagou, V., et al., *Escherichia coli with a self-transferable, multiresistant plasmid coding for metallo-beta-lactamase VIM-1.* Antimicrob Agents Chemother, 2003. **47**(1): p. 395-7.

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

144. Castanheira, M., et al., *Klebsiella pneumoniae Isolate from a New York City Hospital Belonging to Sequence Type 258 and Carrying blaKPC-2 and blaVIM-4.* Antimicrob Agents Chemother, 2016. **60**(3): p. 1924-7.

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

145. Papagiannitsis, C.C., et al., *Biochemical Characterization of VIM-39, a VIM-1-Like Metallo-beta-Lactamase Variant from a Multidrug-Resistant Klebsiella pneumoniae Isolate from Greece.* Antimicrob Agents Chemother, 2015. **59**(12): p. 7811-4.

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

146. Rajabnia, R., et al., *Nosocomial emerging of (VIM1) carbapenemase-producing isolates of Klebsiella pneumoniae in North of Iran.* Iran J Microbiol, 2015. **7**(2): p. 88-93.

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

147. Poirel, L., et al., *Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a Pseudomonas aeruginosa clinical isolate in France.* Antimicrob Agents Chemother, 2000. **44**(4): p. 891-7.

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

148. Schneider I, et al. Detection of CMY-99, a novel acquired AmpC-Type β-lactamase, and VIM-1 in Proteus mirabilis isolates in Bulgaria. Antimicrob Agents Chemother. 2014;58(1):620-1.

Molecular Epi *P. mirabilis* (PCR): 3 clinical isolates of *P. mirabilis* screened for antibiotic resistance genes including VIM-1 (3).

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.

149. Naas, T., L. Poirel, and P. Nordmann, *Minor Extended-Spectrum b-Lactamases.* Clin Microbiol Infect 2008. **14**(1): p. 42–52.

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

150. Poirel, L., et al., *Molecular and Biochemical Characterization of VEB-1, A Novel Class A Extended-Spectrum b-Lactamase Encoded by an Escherichia coli Integron Gene.* Antimicrob Agents Chemother, 1999. **43**: p. 573–581.

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

151. Naas, T., et al., *Molecular characterization of In50, a class 1 integron encoding the gene for the extended-spectrum beta-lactamase VEB-1 in Pseudomonas aeruginosa.* FEMS Microbiol Lett, 1999. **176**(2): p. 411-9.

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

152. Latifpour, M., A. Gholipour, and M.S. Damavandi, *Prevalence of Extended-Spectrum Beta-Lactamase-Producing Klebsiella pneumoniae Isolates in Nosocomial and Community-Acquired Urinary Tract Infections.* Jundishapur J Microbiol, 2016. **9**(3): p. e31179.

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

153. Naas, T., et al., *Integron-located VEB-1 extended-spectrum beta-lactamase gene in a Proteus mirabilis clinical isolate from Vietnam.* J Antimicrob Chemother, 2000. **46**(5): p. 703-11.

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

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