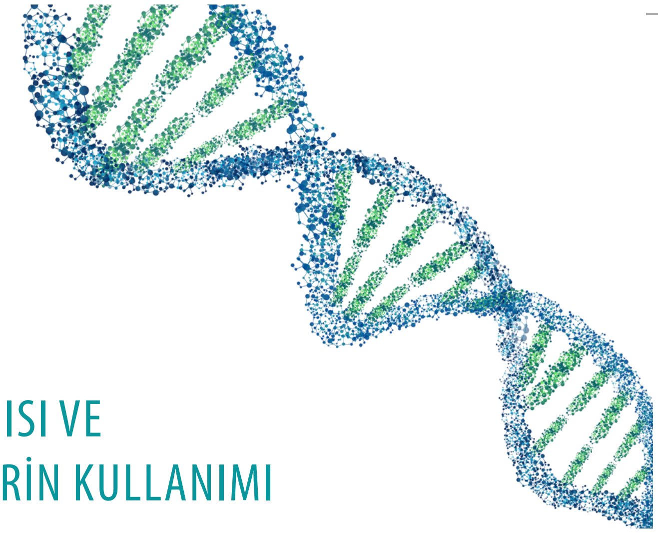

BÖLÜM 44

GIDA KAYNAKLI ENFEKSİYONLARIN TANISI VE KONTROLÜNDE MOLEKÜLER YÖNTEMLERİN KULLANIMI

Mehmet İLKTAÇ[1]
Betigül ÖNGEN[2]

Giriş

Dünya genelinde her yıl yaklaşık olarak 10 kişiden birinde görüldüğü tahmin edilen ve çeşitli bakteri, virüs, parazit, toksin veya kimyasal ile kontamine olmuş su ve gıdaların tüketilmesi ile gelişen gıda kaynaklı hastalıkların büyük bir çoğunluğundan ishalli hastalıklara neden olan enfeksiyon etkenleri sorumludur. Gıda kaynaklı bakteriler neden oldukları enfeksiyonlar aracılığıyla hemolitik üremik sendrom ve Guillain-Barré sendromu gibi ciddi komplikasyonlara ve özellikle gelişmekte olan ülkelerde ölümlere neden olabilmektedir. Bu enfeksiyonlar, ölüme ek olarak sağlık merkezine başvuru ve hastaneye yatışa bağlı sağlık hizmeti harcamaları, erken ölüme bağlı giderler ve iş kayıpları aracılığıyla ülke ekonomisine getirdikleri yük açısından ciddi bir halk sağlığı tehdididir [1].

Prevalansları ve önem dereceleri ülkeden ülkeye değişiklik göstermekle birlikte, dünya genelinde bu enfeksiyonlardan sıklıkla izole edilen bakteriler arasında *Campylobacter, Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Aeromonas* ve *Plesiomonas* türleri ile başta Shiga toksin oluşturan *Escherichia coli* (STEC) olmak üzere diyare etkeni *E. coli* patotipleri [Enteropatojenik *E. coli* (EPEC), Enterotoksijenik *E. coli* (ETEC), Enteroinvaziv *E. coli* (EIEC), Enteroagregatif *E. coli* (EAEC)] yer almaktadır [2,3]. Ayrıca, son yıllarda *Clostridioides difficile* enfeksiyonlarının da gıda kaynaklı olabileceği ileri sürülmektedir[4]. Bu nedenle, bu bölümde *C. difficile* enfeksiyonlarına da yer verilmiştir.

Gıda kaynaklı bakteri enfeksiyonlarının laboratuvar tanısında klasik olarak kültür ve kültürden bağımsız testlerden yararlanılmaktadır. Özgüllüğünün oldukça yüksek olması nedeniyle tanıda altın standart olarak kabul edilen geleneksel kültür yöntemi, izole edilen bakterilerin antibiyotik duyarlılıklarının araştırılmasına, moleküler yöntemlerle tiplendirilerek bölgesel, ulusal veya uluslararası düzeyde salgınlarla ilişkilendirilmesine ve epidemiyolojik açıdan ileri incelemelerin (rezervuar ve

---

[1] Yrd. Doç. Dr., Doğu Akdeniz Üniversitesi, Eczacılık Fakültesi, mehmet.ilktac@emu.edu.tr,
[2] Prof. Dr., İstanbul Üniversitesi, İstanbul Tıp Fakültesi, Tıbbi Mikrobiyoloji AD., ongenb@gmail.com,

birlikte gıda kaynaklı bakterilerin alt tip düzeyinde tiplendirilmesi için uygun değildir [67,83]. Belirli bir bakteriye özgül olan yüzlerce gen bölgesinin çok sayıda farklı primer çifti aracılığıyla çoğaltılarak dizilenmesi esasına dayanan "yüksek düzey multipleks amplikon dizi analizi", *Salmonella* cinsi bakterilerin sürveyansında ümit vadetmektedir. Ancak, dışkı örneğinde hedef bakteri ile genetik benzerlik gösterebilen çok sayıda normal flora elemanı mevcuttur. İhtiyaç duyulan yüzlerce farklı primerin tasarlanabilmesi için normal flora elemanlarınkinden farklılık gösteren hedef dizilerin bulunmasının zor olması, bu yöntemin halk sağlığı sürveyansı açısından dışkı örneklerinde uygulanabilmesini kısıtlamaktadır [81].

Metagenomik yaklaşımlardan bir diğeri olan "shotgun" dizi analizi, hedef gözetmeksizin örnekte bulunan nükleik asitlerin tamamının dizilenmesine imkân tanır [67]. Bu yaklaşım, bakteriye özgül değildir ve etkenle ilgili herhangi bir bilgiye gereksinim duymadan örnekte bulunan herhangi bir mikroorganizmaya ait gen dizilerini saptayabilir [81,82]. "Shotgun" dizi analizinin, klasik tanı yöntemleriyle saptanamayan gıda kaynaklı patojenlerin tanısında, karışık enfeksiyonların saptanmasında ve gıda kaynaklı salgınların araştırılmasında başarılı olduğu çeşitli çalışmalarda gösterilmiştir [84,85]. Ancak, patojen bakterilerin dışkı örneğinde bulunan normal flora elemanlarına kıyasla çok daha az sayıda bulunması nedeniyle bu yöntemin duyarlılığı düşük olabilmektedir. Saptama limitinin 10^4-10^5 cfu/ml olduğu bildirilen "shotgun" dizi analizinin duyarlılığının %67'ye kadar düşebildiği bildirilmektedir [65,86]. Bu yöntemin önemli bir diğer dezavantajı ise sonuçların DNA ekstraksiyonu ve kütüphane hazırlanması gibi çeşitli laboratuvar tekniklerinden ve kullanılan dizi analizi teknolojisi, biyoinformatik analiz sistemleri ve referans veri tabanlarından etkilenmesidir. Bu parametrelerin henüz standardize edilmemiş olması, "shotgun" dizi analizinin gıda kaynaklı bakterilerin tanısında ve rutin sürveyansında kullanımını kısıtlamaktadır [65,82,83].

"Shotgun" dizi analizi, dışkı veya gıda gibi herhangi bir örnekte bulunan direnç genlerinin tamamına yakınının saptanabilmesine de olanak sağlar. Ancak, patojen bakterilerle birlikte çok sayıda diğer bakterilerin de barındığı dışkı veya gıda gibi örneklerde saptanan direnç genleri çoğunlukla bireysel olarak bakterilerle ilişkilendirilemez. Bu nedenle, metagenomik yaklaşımın gıda kaynaklı bakterilere ait antimikrobik direnç sürveyansında tek başına kullanılması henüz mümkün değildir [65].

Sonuç

Kaydedilen ilerlemelerle bir paradigma kaymasına yol açabilecek potansiyele sahip olan moleküler yöntemlerin halen önemli kısıtlılıklarının bulunması gıda kaynaklı enfeksiyonların tanısı ve kontrolünde geleneksel kültür yöntemlerinin yerini almasını şu an için engellese de bu yöntemlerin çok yakın bir gelecekte hastalıkların tanısı ve salgın yönetiminde de ön plana geçeceği açıktır.

Kaynaklar

1. WHO estimates of the global burden of foodborne diseases. Foodborne disease burden epidemiology reference group 2007-2015. World Health Organization Press; 2015 Geneva.
2. Humphries RM, Linscott AJ. Practical guidance for clinical microbiology laboratories: Laboratory diagnosis of bacterial gastroenteritis. Clin Microbiol Rev 2015;28(1): 3-31.
3. Amjad M. An overview of molecular methods in the diagnosis of gastrointestinal infectious diseases. Int J Microbiol 2020;2020: 8135724.
4. Lund BM, Peck MW. A possible route for foodborne transmission of *Clostridium difficile*? Foodborne Pathog Dis 2015;12(3): 177-182.
5. Vila J. New molecular diagnostic tools in traveller's diarrhea. J Travel Med 2017;24(Suppl1): S23-S28.
6. Zboromyrska Y, Vila J. Advanced PCR-based molecular diagnosis of gastrointestinal infections: challenges and opportunities. Expert Rev Mol Diagn 2016;16(6): 631-40.
7. Fitzgerald C, Patrick M, Gonzalez A, Akin J, Polage CR, Wymore K, et al. Multicenter evaluation of clinical diagnostic methods for detection and isolation of *Campylobacter* spp. from stool. J Clin Microbiol 2016;54(5): 1209-15.
8. Couturier MR. Revisiting the roles of culture and culture-independent detection tests for *Campylobacter.* J Clin Microbiol 2016;54(5): 1186-1188.
9. Gould LH. Recommendations for diagnosis of shiga toxin-producing *Escherichia coli* infections by clinical laboratories. MMWR Recomm Rep 2009;58(RR-12): 1-14.

10. Tarr GA, Lin CY, Vandermeer B, Lorenzetti DL, Tarr PI, Chui L, et al. Diagnostic test accuracy of commercial tests for detection of shiga toxin-producing *Escherichia coli*: A systematic review and meta-analysis. Clin Chem 2020;66(2): 302-315.
11. Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. Clin Microbiol Rev 2018;31(1): e00024-17.
12. Maldonado-Garza HJ, Garza-Gonzalez E, Bocanegra-Ibanias P, Flores-Treviňo S. Diagnostic syndromic multiplex approaches for gastrointestinal infections. Expert Rev Gastroenterol Hepatol 2021;15(7): 743-57.
13. Zhang H, Morrison S, Tang YW. Multiplex polymerase chain reaction tests for detection of pathogens associated with gastroenteritis. Clin Lab Med 2015;35(2): 461-86.
14. Mengelle C, Mansuy JM, Prere MF, Grouteau E, Claudet I, Kamar N, et al. Simultaneous detection of gastrointestinal pathogens with a multiplex Luminex-based molecular assay in stool samples from diarrhoeic patients. Clin Microbiol Infect 2013;19(10): E458-65.
15. Huang RSP, Jhonson CL, Pritchard L, Hepler R, Ton TT, Dunn JJ. Performance of the Verigene® enteric pathogens test, Biofire FilmArray™ gastrointestinal panel and Luminex xTAG® gastrointestinal pathogen panel for detection of common enteric pathogens. Diagn Microbiol Infect Dis 2016;86(4): 336-339.
16. Zhan Z, Guo J, Xiao Y, He Z, Xia X, Huang Z, et al. Comparison of BioFire FilmArray gastrointestinal panel versus Luminex xTAG Gastrointestinal Pathogen Panel (xTAG GPP) for diarrheal pathogen detection in China. Int J Infect Dis 2020;99: 414-420.
17. Yalamanchili H, Dandachi D, Okhuysen PC. Use and interpretation of enteropathogen multiplex nucleic acid amplification tests in patients with suspected infectious diarrhea. Gastroenterol Hepatol (NY) 2018;14(11): 646-652.
18. Teh R, Tee WD, Tan E, Fan K, Koh CJ, Tambyah PA, et al. Review of the role of gastrointestinal multiplex polymerase chain reaction in the management of diarrheal illness. J Gastroenterol Hepatol 2021;36(12): 3286-3297.
19. Chang LJ, Hsiao CJ, Chen B, Liu TY, Ding J, Hsu WT, et al. Accuracy and comparison of two rapid multiplex PCR tests for gastroenteritis pathogens: A systematic review and meta-analysis. BMJ Open Gastroenterol 2021;8(1): e000553.
20. Deng J, Luo X, Wang R, Jiang L, Ding X, Hao W, et al. A comparison of Luminex xTAG® Gastrointestinal Pathogen Panel (xTAG GPP) and routine tests for the detection of enteropathogens circulating in Southern China. Diagn Microbiol Infect Dis 2015;83(3): 325-330.
21. Duong VT, Phat VV, Tuyen HT, Dung TTN, Trung PD, Minh PV, et al. Evaluation of Luminex xTAG Gastrointestinal Pathogen Panel Assay for detection of multiple diarrheal pathogens in fecal samples in Vietnam. J Clin Microbiol 2016;54(4): 1094-100.
22. Khare R, Espy MJ, Cebelinski E, Bozrud D, Sloan LM, Cunningham SA, et al. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. J Clin Microbiol 2014;52(10): 3667-3673.
23. Claas EC, Burnham CAD, Mazzulli T, Templeton K, Topin F. Performance of the xTAG® Gastrointestinal Pathogen Panel, a multiplex molecular assay for simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis. J Microbiol Biotechnol 2013;23(7): 1041-1045.
24. Halligan E, Edgeworth J, Bisnauthsing K, Bible J, Cliff P, Aarons E, et al. Multiplex molecular testing for management of infectious gastroenteritis in a hospital setting: A comparative diagnostic and clinical utility study. Clin Microbiol Infect 2014;20(8): O460-467.
25. Yoo J, Park J, Lee HK, Yu JK, Lee GD, Park KG, et al. Comparative evaluation of Seegene Allplex Gastrointestinal, Luminex xTAG Gastrointestinal Pathogen Panel, and BD MAX Enteric Assays for detection of gastrointestinal pathogens in clinical stool specimens. Arch Pathol Lab Med 2019;143(8): 999-1005.
26. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, et al. Multicenter evaluation of the BioFire FilmArray Gastrointestinal Panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol 2015;53(3): 915-925.
27. Jo SJ, Kang HM, Kim JO, Cho H, Heo W, Yoo IY, et al. Evaluation of the BioFire Gastrointestinal Panel to detect diarrheal pathogens in pediatric patients. Diagnostics (Basel) 2021;12(1): 34.
28. Kosai K, Suzuki H, Tamai K, Okada Y, Akamatsu N, Ueda A, et al. Multicenter evaluation of Verigene Enteric Pathogens Nucleic Acid Test for detection of gastrointestinal pathogens. Sci Rep 2021;11(1): 3033.
29. Hannet I, Engsbro AL, Pareja J, Schneider UV, Lisby JG, Pružinec-Popović B, et al. Multicenter evaluation of the new QIAstat Gastrointestinal Panel for the rapid syndromic testing of acute gastroenteritis. Eur J Clin Microbiol Infect Dis 2019;38(11): 2103-2112.
30. Engberg J, Vejrum LK, Madsen TV, Nielsen XC. Verification of analytical bacterial spectrum of QIAstat-Dx® GI V2 and Novodiag® Bacterial GE+ V2-0 diagnostic panels. J Antimicrob Chemother 2021;76(Suppl 3): iii50-iii57.
31. Coupland LJ, McElarney I, Meader E, Cowley K, Alcock L, Naunton J, et al. Simultaneous detection of viral and bacterial enteric pathogens using the Seeplex® Diarrhea ACE detection system. Epidemiol Infect 2013;141(10): 2111-2121.
32. Onori M, Coltella L, Mancinelli L, Argentieri M, Menichella D, Villani A, et al. Evaluation of a multiplex PCR assay for simultaneous detection of bacterial and viral enteropathogens in stool samples of paediatric patients. Diagn Microbiol Infect Dis 2014;79(2): 149-154.
33. Martín-Díaz A, Muñoz-Gallego I, Lora D, Pérez de Ayala A, Orellana-Miguel MA. Evaluation of the new multiplex real-time PCR technique Allplex GI-Bacteria I and Allplex GI-Bacteria II assay, Seegene in the diagnosis of gastrointestinal diseases of bacterial etiology. 27. ECCMID Kongresi, 22-25 Nisan 2017, Viyana.
34. Harrington SM, Buchan BW, Doern C, Fader R, Ferraro MJ, Pillai DR, et al. Multicenter evaluation of the BD Max Enteric Bacterial Panel PCR assay for rapid detection of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. (*C. jejuni* and *C. coli*), and Shiga toxin 1 and 2 genes. J Clin Microbiol 2015;53(5): 1639-1647.
35. Berenger BM, Chui L, Ferrato C, Lloyd T, Li V, Pillai DR. Performance of four commercial real-time PCR assays for the detection of bacterial enteric pathogens in clinical samples. Int J Infect Dis 2022;114: 195-201.
36. Biswas JS, Al-Ali A, Rajput P, Smith D, Goldenberg SD. A

parallel diagnostic accuracy study of three molecular panels for the detection of bacterial gastroenteritis. Eur J Clin Microbiol Infect Dis 2014;33(11): 2075-2081.

37. Knabl L, Grutsch I, Orth-Höller D. Comparison of the BD MAX® Enteric Bacterial Panel assay with conventional diagnostic procedures in diarrheal stool samples. Eur J Clin Microbiol Infect Dis 2016;35: 131-136.
38. Simner PJ, Pillai DR, Oethinger M, Yogev R, Stellrecht K, Mortensen JE. Multisite evaluation of the BD MAX™ Extended Enteric Bacterial Panel for the detection of *Yersinia enterocolitica*, Enterotoxigenic *E. coli*, *Vibrio* and *Plesiomonas shigelloides* from stool specimens. J Clin Microbiol 2017;55(11): 3258-3266.
39. Valledor S, Valledor I, Gil-Rodríguez MC, Seral C, Castillo J. Comparison of several real-time PCR kits versus a culture-dependent algorithm to identify enteropathogens in stool samples. Sci Rep 2020;10(1): 4301.
40. Buchan BW, Olson WJ, Pezewski M, Marcon MJ, Novicki T, Uphoff TS. Clinical evaluation of a real-time PCR assay for identification of *Salmonella*, *Shigella*, *Campylobacter* (*Campylobacter jejuni* and *C. coli*), and shiga toxin-producing *Escherichia coli* isolates in stool specimens. J Clin Microbiol 2013;51(12): 4001-4007.
41. Torres-Miranda D, Akselrod H, Karsner R, Secco A, Silva-Cantillo D, Siegel MO, et al. Use of BioFire FilmArray gastrointestinal PCR panel associated with reductions in antibiotic use, time to optimal antibiotics, and length of stay. BMC Gastroenterol 2020;20(1): 246.
42. Freeman K, Tsertsvadze A, Taylor-Phillips S, McCarthy N, Mistry H, Manuel R, et al. Agreement between gastrointestinal panel testing and standard microbiology methods for detecting pathogens in suspected infectious gastroenteritis: Test evaluation and meta-analysis in the absence of a reference standard. PLoS One 2017;12(3): e0173196.
43. Spina A, Kerr KG, Cormican M, Barbut F, Eigentler A, Zerva L, et al. Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicentre study of community-acquired gastroenteritis. Clin Microbiol Infect 2015;21(8): 719-728.
44. Axelrad JE, Freedberg DE, Whittier S, Greendyke W, Lebwohl B, Green DA. Impact of gastrointestinal panel implementation on health care utilization and outcomes. J Clin Microbiol 2019;57(3): e01775-18.
45. Macfarlane-Smith LR, Ahmed S, Wilcox MH. Molecular versus culture-based testing for gastrointestinal infection. Curr Opin Gastroenterol 2018;34(1): 19-24.
46. Iwamoto M, Huang JY, Cronquist AB, Medus C, Hurd S, Zansky S, et al. Bacterial enteric infections detected by culture independent diagnostic tests-Foodnet, United States, 2012-2014. MMWR Morb Mortal Wkly Rep 2015;64(9): 252-257.
47. Beal SG, Tremblay EE, Toffel S, Velez L, Rand KH. A gastrointestinal PCR panel improves clinical management and lowers health care costs. J Clin Microbiol 2017;56(1): e01457-17.
48. Machiels JD, Cremers AJH, van Bergen-Verkuyten MCGT, Paardekoper-Strijbosch SJM, Frijns KCJ, Wertheim HFL, et al. Impact of the BioFire FilmArray gastrointestinal panel on patient care and infection control. PLoS One 2020;15(2): e0228596.
49. Cybulski RJ Jr, Bateman AC, Bourassa L, Bryan A, Beail B, Matsumoto J, et al. Clinical impact of a multiplex gastrointestinal polymerase chain reaction panel in patients with acute gastroenteritis. Clin Infect Dis 2018;67(11): 1688-1696.
50. Abbott AN, Fang FC. Clinical impact of multiplex syndromic panels in the -diagnosis of bloodstream, gastrointestinal, respiratory, and central nervous system infections. Clin Microbiol Newsl 2017;39(17): 133-142.
51. Goldenberg SD, Bacelar M, Brazier P, Bisnauthsing K, Edgeworth JD. A cost benefit analysis of the Luminex xTAG Gastrointestinal Pathogen Panel for detection of infectious gastroenteritis in hospitalised patients. J Infect 2015;70(5): 504-511.
52. Dumkow LE, Worden LJ, Rao SN. Syndromic diagnostic testing: A new way to approach patient care in the treatment of infectious diseases. J Antimicrob Chemother 2021;76(Suppl 3): iii4–iii11.
53. Riddle MS, DuPont HL, Connor BA. ACG clinical guideline: Diagnosis, treatment, and prevention of acute diarrheal infections in adults. Am J Gastroenterol 2016;111(5): 602-6022.
54. Marder EP, Cieslak PR, Cronquist AB, Dunn J, Lathrop S, Rabatsky-Ehr T, et al. Incidence and trends of infections with pathogens transmitted commonly through food and the effect of increasing use of culture-independent diagnostic tests on surveillance - Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 2013-2016. MMWR Morb Mortal Wkly Rep 2017;66(15): 397-403.
55. Frickmann H, Schwarz NG, Rakotozandrindrainy R, May J, Hagen RM. PCR for enteric pathogens in high-prevalence settings. What does a positive signal tell us? Infect Dis (Lond) 2015;47(7): 491-498.
56. Enserink R, Scholts R, Bruijning-Verhagen P, Duizer E, Vennema H, de Boer R, et al. High detection rates of enteropathogens in asymptomatic children attending day care. PLoS One 2014;9(2): e89496.
57. Bonacorsi S, Visseaux B, Bouzid D, Pareja J, Rao SN, Manissero D, et al. Systematic review on the correlation of quantitative PCR cycle threshold values of gastrointestinal pathogens with patient clinical presentation and outcomes. Front Med (Lausanne);8: 711809.
58. Whole genome sequencing for foodborne disease surveillance: Landscape paper. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO.
59. Wei X, Zhao X. Advances in typing and identification of foodborne pathogens. Curr Opin Food Sci 2021;37: 52-57.
60. Carleton HA, Gerner-Smidt P. Whole-genome sequencing is taking over foodborne disease surveillance. Microbe 2016;11(7): 311-317.
61. Ribot EM, Freeman M, Hise KB, Gerner-Smidt P. PulseNet: Entering the age of next-generation sequencing. Foodborne Pathog Dis 2019;16(7): 451-456.
62. Fabre L, Zhang J, Guigon G, Hello SL, Guibert V, Accou-Demartin M, et al. CRISPR typing and subtyping for improved laboratory surveillance of *Salmonella* infections. PLoS One 2012;7(5): e36995.
63. Kubota KA, Wolfgang WJ, Baker DJ, Boxrud D, Turner L, Trees E, et al. PulseNet and the changing paradigm of laboratory-based surveillance for foodborne diseases. Public Health Rep 2019;134(2_suppl): 22S-28S.
64. Kovac J, Dudley EG, Nawrocki EM, Yan R, Chung T. Whole genome sequencing: The impact on foodborne outbreak

investigations. Comprehensive Foodomics 2021;1: 147-159.

65. EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, et al. Scientific opinion on the whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms. EFSA Journal 2019;17(12): 5898.
66. Moran-Gilad J. Whole genome sequencing (WGS) for food-borne pathogen surveillance and control – taking the pulse. Euro Surveill 2017;22(23): 30547.
67. Besser J, Carleton H, Goering R, Gerner-Smidt P. The impact of molecular diagnostics on surveillance of foodborne infections, pp. 235-244. In: Persing DH, Tenover FC, Hayden RT, Leven M, Miller MB, Nolte FS, et al (eds), Molecular Microbiology: Diagnostic Principles and Practice. 2016, 3rd ed. ASM Press, Washington.
68. Uelze L, Grützke J, Borowiak M, Hammerl JA, Juraschek K, Deneke C, et al. Typing methods based on whole genome sequencing data. One Health Outlook 2020;2: 3.
69. Hendriksen RS, Pedersen SK, Leekitcharoenphon P, Malorny B, Borowiak M, Battisti A, et al. Final report of ENGAGE - Establishing next generation sequencing ability for genomic analysis in Europe. EFSA Supporting Publication 2018; EN-1431.
70. Uelze L, Borowiak M, Deneke C, Szabó I, Fischer J, Tausch SH, et al. Performance and accuracy of four open-source tools for *in silico* serotping of *Salmonella* spp. Based on whole-genome short-read sequencing data. Appl Environ Microbiol 2020;86(5): e02265-19.
71. Joensen KG, Tetzschner AM, Iguchi A, Aarestrup FM, Scheutz F. Rapid and easy *in silico* serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data. J Clin Microbiol 2015;53(8): 2410-2426.
72. Ingle DJ, Valcanis M, Kuzevski A, Tauschek M, Inouye M, Stinear T, et al. *In silico* serotyping of *E. coli* from short read data identifies limited novel O-loci but extensive diversity of O:H serotype combinations within and between pathogenic lineages. Microb Genom 2016;2(7): e000064.
73. Yachison CA, Yoshida C, Robertson J, Nash JHE, Kruczkiewicz P, Taboada EN, et al. The validation and implications of using whole genome sequencing as a replacement for traditional serotyping for a national *Salmonella* reference laboratory. Front Microbiol 2017;8: 1044.
74. Collineau L, Boerlin P, Carson CA, Chapman B, Fazil A, Hetman B, et al. Integrating whole-genome sequencing data into quantitative risk assessment of foodborne antimicrobial resistance: A review of opportunities and challenges. Front Microbiol 2019;10: 1107.
75. Whitehouse CA, Young S, Li C, Hsu CH, Martin G, Zhao S. Use of whole-genome sequencing for *Campylobacter* surveillance from NARMS retail poultry in the United States in 2015. Food Microbiol 2018;73: 122-128.
76. Rokney A, Valinsky L, Vranckx K, Feldman N, Agmon V, Moran-Gilad J, et al. WGS-based prediction and analysis of antimicrobial resistance in *Campylobacter jejuni* isolates from Israel. Front Cell Infect Microbiol 2020;10: 365.
77. Hodges LM, Taboada EN, Koziol A, Mutschall S, Blais BW, Inglis GD, et al. Systematic evaluation of whole-genome sequencing based prediction of antimicrobial resistance in *Campylobacter jejuni* and *C. coli.* Front Microbiol 2021;12: 776967.
78. Cooper AL, Low AJ, Koziol AG, Thomas MC, Leclair D, Tamber S, et al. Systematic evaluation of whole genome sequence-based predictions of *Salmonella* serotype and antimicrobial resistance. Front Microbiol 2020;11: 549.
79. McDermott PF, Tyson GH, Kabera C, Chen Y, Li C, Folster JP, et al. Whole-genome sequencing for detecting antimicrobial resistance in nontyphoidal *Salmonella.* Antimicrob Agents Chemother 2016;60(9): 5515-5520.
80. Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, et al. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Clin Microbiol Infect 2017;23(1): 2-22.
81. Carleton HA, Besser J, Williams-Newkirk AJ, Huang A, Trees E, Gerner-Smidt P. Metagenomic approaches for public health surveillance of foodborne infections: Opportunities and challenges. Foodborne Pathog Dis 2019;16(7): 474-479.
82. Forbes JD, Knox NC, Ronholm J, Pagotto F, Reimer A. Metagenomics: The next culture-independent game changer. Front Microbiol 2017;8: 1069.
83. Andersen SC, Hoorfar J. Surveillance of foodborne pathogens: Towards diagnostic metagenomics of fecal samples. Genes (Basel) 2018;9(1): 14.
84. Joensen KG, Engsbro ALØ, Lukjancenko O, Kaas RS, Lund O, Westh H, et al. Evaluating next-generation sequencing for direct clinical diagnostics in diarrhoeal disease. Eur J Clin Microbiol Infect Dis 2017;36: 1325-1338.
85. Huang AD, Luo C, Pena-Gonzalez A, Weigand MR, Tarr CL, Konstantinidis KT. Metagenomics of two severe foodborne outbreaks provides diagnostic signatures and signs of coinfection not attainable by traditional methods. Appl Environ Microbiol 2017;83(3): e02577-16.
86. Loman NJ, Constantinidou C, Christner M, Rohde H, Xhan JZM, Quick J, et al. A culture-independent sequence-based metagenomics approach to the investigation of an outbreak of shiga-toxigenic *Escherichia coli* O104:H4. JAMA 2013;309(14): 1502-1510.