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Molecular typing methods for major food-borne microbiological hazards and their use for attribution modelling, outbreak investigation and scanning surveillance

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Abstract. The paper presents a recent Opinion of the Biohaz Panel of EFSA, which provides an evaluation of molecular typing methods that can be applied to the food-borne pathogens *Salmonella*, *Campylobacter*, Shiga toxin-producing *Escherichia coli* and *Listeria monocytogenes*. This evaluation is divided in two parts. First, commonly used molecular typing methods are assessed against a set of predefined criteria relating to discriminatory power, reproducibility, repeatability and current or potential suitability for international harmonisation. Secondly, the methods are evaluated regarding their appropriateness for use in different public health-related applications. These applications include outbreak detection and investigation, attribution modelling, the potential for early identification of food-borne clones with epidemic potential and the integration of the resulting data in risk assessment. The results of these evaluations provide updated insights into the potential use of molecular characterisation methods, including whole genome sequencing technologies, in microbial food safety. Recommendations are also made in order to encourage a holistic and structured approach to the use of molecular characterisation methods for food-borne pathogens; in particular, on the importance of structured co-ordination at international level to help overcome current limitations in harmonisation of data analysis and interpretation.

Keywords. Genotyping – Molecular typing – Whole genome sequencing – Outbreak – Source attribution – Epidemic potential.

Les méthodes de typage moléculaire pour les principaux dangers microbiologiques liés aux aliments et leur utilisation pour la modélisation de l'attribution, l'investigation des flambées épidémiques et la surveillance passive.

Résumé. *Cet article présente un avis récent du groupe scientifique de l'EFSA sur les dangers biologiques (BIOHAZ), qui évalue les méthodes de typage moléculaire pouvant être appliquées aux pathogènes liés aux aliments tels que* Salmonella, Campylobacter, Escherichia coli *Shigatoxigène (STEC) et* Listeria monocytogenes. *Cette évaluation est divisée en deux parties. D'abord, les méthodes de typage moléculaire couramment utilisées sont évaluées selon un ensemble de critères prédéfinis concernant le pouvoir de discrimination, la reproductibilité, la répétabilité et l'adaptation actuelle ou potentielle à l'harmonisation internationale. En deuxième lieu, les méthodes sont évaluées en vue de leur utilisation dans différentes applications liées à la santé publique. Parmi ces applications figurent la détection et l'investigation des flambées épidémiques, la modélisation de l'attribution, les possibilités d'identification précoce des souches*

d'origine alimentaire à potentiel épidémique et l'intégration des données résultantes dans l'évaluation des risques. Les résultats de ces évaluations permettent une vision actualisée de l'utilisation et du potentiel des méthodes de caractérisation moléculaire, y compris les technologies de séquençage du génome entier, pour la sécurité microbienne des aliments. Des recommandations sont également formulées afin d'encourager une approche holistique et structurée de l'utilisation des méthodes de caractérisation moléculaire concernant les pathogènes présents dans les aliments ; en particulier est soulignée l'importance d'une coordination structurée au niveau international afin de surmonter les limitations actuelles quant à l'harmonisation de l'analyse et l'interprétation des données.

Mots-clés. *Génotypage – Typage moléculaire – Séquençage du génome entier – Flambée épidémique – Attribution des sources – Potentiel épidémique.*

I – Introduction

Molecular typing can be defined as the classification of microorganisms on the basis of variation in the genotype, and/or the presence or absence of specific genes, such as those which may contribute to the pathogenicity of the organism or to its ability to survive in less favourable environments (Hallin et al., 2012). 'Genotype' has been defined as the genetic constitution of an organism, as assessed by a molecular method (van Belkum *et al*., 2007).

According to the European Centre for Disease Prevention and Control (ECDC), molecular typing refers to the application of laboratory methods capable of characterizing, discriminating and indexing subtypes of microorganisms. Molecular typing of pathogens that cause infectious diseases complements traditional epidemiological surveillance by providing appropriate discriminatory analyses to: (i) allow the rapid and early detection of outbreaks; (ii) investigate transmission chains; (iii) determine the relatedness of strains; and, (iv) detect the emergence of antimicrobial resistance and new evolving pathogenic strains. Molecular typing can also support studies to trace-back the source of an outbreak and identify new risk factors, by linking isolates more accurately to epidemiological and clinical data (ECDC, 2007 and 2013).

Genetic methods for bacterial typing have progressively replaced phenotypic assays during the last two decades, even though the phenotypic methods are still widely used by reference laboratories for routine surveillance and outbreak detection, as reported in an EU-wide survey (EFSA, 2009). The current practice is to use a combination of different phenotypic and genotypic typing methods.

During the last three decades, a large number of genotyping methods have been developed and applied in various contexts, mostly by research institutions or reference laboratories dealing with local or national outbreaks. Difficulties in standardisation and harmonisation of the results have often made data difficult to share. For some methods, standardisation and harmonisation has been developed to a degree that has made application of the methods suitable for wider international use (e.g. Pulsenet International).

Recently, the Biohaz Panel of EFSA has adopted an opinion (EFSA, 2013) in which the main molecular typing methods that are currently used and prospective methods for epidemiological typing of the main food-borne bacteria (*Campylobacter*, *Salmonella*, Shiga-toxin producing *Escherichia coli* (STEC) and *Listeria* by national and international reference laboratories are considered. These were evaluated in terms of: (i) discriminatory power (i.e. degree of discrimination between strains of different genotype); (ii) reproducibility and repeatability (i.e. consistency of results within and between laboratories, and over time); (iii) current international harmonisation (i.e. status with regard to availability and use of standard operational procedures; external quality assurance systems, harmonised nomenclature and data management tools), and, (iv) the potential for future international harmonisation in situations where any of the sub-criteria under (iii) may not be currently harmonised.

The document highlights that all bacteria are subject to genetic change (e.g. in response to environmental stress and human interventions such as antimicrobial or heavy metal use or vaccination, or by natural genetic drift), by mutation or by acquisition or loss of genetic elements. These changes can be followed by clonal expansion in the case of biologically successful organisms. Ongoing evolution driven by genetic change and selection has given rise to organisms that are able to exploit and expand into novel niches and extend their host range. Such evolution may also be linked to the emergence of various 'epidemic' strains of pathogens, such as *Salmonella*, in combination with other biological factors and epidemiological opportunities for dissemination. The molecular characteristics of organisms provide markers for investigation of outbreaks, attribution studies, and assessment of potential virulence or epidemic potential. The Opinion also points out that even with high-resolution molecular approaches, up to and including WGS analysis, it is not possible to establish how closely two isolates are related without an appreciation of the structure and diversity of the bacterial population in question. Further, to properly evaluate typing methodologies, data from strain characterisation should be linked with epidemiological data and, as far as is possible, the strain selection must be unbiased and statistically representative of the population. International harmonisation of molecular characterisation outputs by means of standardisation or appropriate quality control procedures is essential. This includes controlling the accuracy of production of DNA sequences from WGS and the further interpretations of annotation pipelines.

II – Molecular serotyping

Molecular serotyping describes methods developed to identify serotypes of organisms by analysing DNA. There are several ways in which DNA-analysis can be used to achieve this. The most common methodology uses either one of these two key principles: (a) examination of the genetic loci known to produce the serologically reactive components used in traditional serotyping; or, (b) examination of variations in the genome, which are indirectly associated with known serovars or serotypes. These variations may include various kinds of polymorphous regions, as long as they show a strong association to the traditional serovars/serotypes.

Molecular serotyping is considered to provide a low to moderate discriminatory capability. This is normally similar or marginally higher than traditional serotyping as sub-types can often be recognised within serotypes. 'Reproducibility and repeatability' are high, but may be reduced if large arrays are used, due to the complexity of the technology. 'Internationally harmonised standards' for molecular serotyping are not in place except for *L. monocytogenes*; nevertheless, the existing software tools could be employed at international level. Molecular serotyping is based on a well-known and implemented methodology, and thus has a high capability for 'future international harmonisation'. Molecular serotyping will, in most instances, provide results within a day from receiving the isolate. Molecular serotyping using MLST derived from whole genome sequencing is likely to be increasingly used in future, replacing the array-based methods that are in current use.

III – Restriction Fragment Length Polymorphism (RFLP) analysis

In RFLP, a target DNA sequence known to show polymorphism between strains of a bacterial species, is cut with one or more restriction endonucleases to generate fragments of varying length. The earliest versions of the RFLP method involved several time-consuming steps. The whole process could in some cases take up to four weeks to produce an interpretable result.

In PCR-RFLP typing the target sequence is amplified at high annealing temperatures to maximise stringency. The amplified product is cut with one or more restriction endonucleases and the type is determined by comparing RFLP patterns after gel electrophoresis. PCR-RFLP typing has provided limited discrimination.

When RFLP analysis is directed at genes encoding ribosomal ribonucleic acid (rRNA) the method is usually referred to as 'Ribotyping'. Ribotyping s has successfully been automated, and fully automated ribotyping is commonly referred to as 'riboprinting' after the RiboPrinter® commercial system (DuPont Qualicon, Wilmington, DE). Automated riboprinters require minimal input and technical skill by the operator, but the cost of equipment is high, so this method is largely used by commercial food companies.

RFLP analysis may be regarded as providing a moderate to high 'discriminatory capability' for at least some of the four pathogens considered in this manuscript. Within and between laboratories' reproducibility and repeatability is low to moderate for PCR-RLFP and traditional ribotyping, but high in the case of fully automated ripoprinting systems. At present, the riboprinting platform provided by DuPont Qualicon® appears to be the only RLFP typing that provides for 'internationally harmonised standards'. Nevertheless, RLFP typing tools other than riboprinting also may have the 'potential for international harmonisation' in spite of the current lack of systems operating to achieve this.

IV – Pulsed-Field Gel Electrophoresis (PFGE) analysis

PFGE was first described in 1984 and is currently the most frequently used DNA-based typing method for food-borne bacterial pathogens. The PFGE-method standardization and rigid quality control introduced by PulseNet International has resulted in PFGE becoming the most commonly used method for outbreak identification, surveillance and investigation for a number of important pathogens, in particular *Salmonella*, STEC and *Listeria* (Ribot *et al.,* 2006). Thus, for these pathogens, the performance of new typing methods will be measured against PFGE.

PFGE fingerprinting has a high 'discriminatory power for most pathogens considered, but for the species *Salmonella enterica* there are some notable exceptions, namely *S. typhimurium* DT 104, and *S.* Enteritidis PT 4. For these two, the fact that they are subtypes of a subspecies and their recent emergence has led to a high degree of clonality. The discriminatory power of PFGE depends on the number and distribution of restriction sites throughout the genome, including extra-chromosomal DNA, which define the number and sizes of bands in the profile, and can be increased by using different or combinations of restriction endonucleases. Within and between laboratory 'reproducibility and repeatability' of results, based on the experience gained in the context of PulseNet International and PulseNet Europe, can be high, but the technique may be considered to be laborious and time consuming. PFGE may require several days for completion, with time increasing with the number of restriction enzymes used. 'Harmonised standards' are available, with the exception of a harmonised nomenclature, although for *Salmonella* a harmonised and agreed nomenclature is used within the EU. Nevertheless, achieving a uniform international nomenclature for 'future harmonisation' should be possible.

V – Multiple-Locus Variable number tandem repeat Analysis (MLVA)

All bacterial MLVA-assays simultaneous measure the length of variable number of tandem repeat (VNTR) loci by PCR amplification and electrophoresis, and use this information to create a genotype to distinguish between isolates of the same species.

MLVA has several advantages: it has a high discriminatory power, which can be easily adjusted by inclusion or exclusion of loci to be investigated; handling of pathogenic bacteria is low, which increases laboratory safety; rapidity, as both PCR and electrophoresis times can now be greatly reduced due to improved technology.

MLVA typing has a high discriminatory power for *Salmonella*, STEC and *L. monocytogenes* but not for *Campylobacter*. Only *S. typhimurium* MLVA has so far been validated for international

reproducibility and repeatability, and results indicate high reproducibility and repeatability when strict guidelines and a reference strain collection are used. MLVA allows direct digital storage of results as discrete-character numeric data. For inter-laboratory comparability and the correct assignation of the numeric profile, calibration of measured fragment sizes has to be performed in each laboratory (Larsson *et al.,* 2009). A proposed standardisation scheme also exists for *S.* Enteritidis. Thus, international harmonisation appears well advanced, in particular for *S.* Typhimurium. Furthermore, the potential for future international harmonisation for *Listeria* and STEC, but not for *Campylobacter*, should be possible based on the experience with *S. typhimurium*. MLVA results can be obtained within 24 hours of receiving isolates.

VI – Sequence-based typing methods

1. Single Locus Sequence Typing (SLST)

SLST describes the sequencing of a single gene or genetic locus, which displays enough polymorphism to be used in a typing scheme. Usually one single locus is sequenced and compared between strains to determine the genetic distance. The SLST method thus entails the same operational steps as running Multi locus sequence typing (MLST, see below) the only difference is the number and choice of the target loci. Equipment and analysis software used will in most instances be the same. Sequencing of the *fla*A short variable region (SVR) may be used for typing of *Campylobacter* (Meinersmann *et al.,* 1997). This provides good discrimination within *C. jejuni* and *C. coli*, and an international nomenclature is established (via the pubMLST database). The *fla*A-SVR is often used as an additional locus to the seven MLST loci to improve the discriminatory power of MLST.

SLST has a high discriminatory power for subtyping known STEC STX-producing variants, and moderate capability for *Campylobacter* spp. *fla*A SVR typing. For *Salmonella* and *Listeria*, SLST is not commonly used. Reproducibility and repeatability are considered high but current international harmonisation requires the establishment of international SOPs and EQA procedures, although harmonised nomenclature and data management tools are already in place. These could be developed without major difficulties, so the method could have a high capability for future international harmonisation SLST methodology is well proven, and typing results in most cases will be available with 24 hours.

2. Multi locus sequence typing (MLST)

MLST indexes sequences variation at a number (usually seven) genetic loci distributed around the chromosome (Maiden, 2006). These are ideally housekeeping genes, i.e. genes encoding enzymes that are involved in primary metabolism of the organism in question and which are therefore present in all isolates. Such genes are stable, in that the metabolic function must be conserved. With this method an allelic profile or sequence type (ST) is created for each pathogen. The STs are also assigned unique arbitrary identifiers so that the sequence variation can be summarised as a single number. The existence of web-accessible databases of allele definition, STs and isolate data enables the unambiguous comparison of data collected in different laboratories. A number of analysis approaches can be used to examine structure within MLST datasets and establish relationships among STs which are crucial for identifying membership of higher groups, known as clonal complexes.

The discriminatory power of MLST is moderate to high depending on the pathogen and gene subset typed; usually the discriminatory capability for food-borne pathogens is too low for outbreak investigations and thus additional typing data is needed when used in this context. ‘Reproducibility and repeatability’ are high and current international harmonisation is well advanced, although international SOPs could benefit from standardising an assay for each pathogen, rather than allowing different methodologies to be used.

VII – Whole Genome Sequence (WGS) analysis

Most of the prominent new technologies are the sequence-based. Several versions of new sequencing technologies, employing different principles, are in existence, all of which are designed with the aim of rapid sequencing of whole genomes. An often-used term is 'Next Generation Sequencing' (NGS), which is commonly used to refer to the post-Sanger and Maxam–Gilbert sequencing methods (Struelens and Brisse, 2013).

There are four approaches currently in use: (i) pyrosequencing, exemplified by the Roche 454 platform which can generate longer but fewer reads and with potential miscalling of polynucleotide sequences (this platform is about to be discontinued and can be considered to be redundant); (ii) Illumina sequencing technology, which produces shorter but more sequence reads; (iii) IonTorrent, also produces shorter sequence reads, and with a potential for miscalling polynucleotide tracts; and (iv) the PacBio SMRT sequencing system, which can produce very long sequences and epigenetic features such as DNA methylation, but with relatively high error rates and cost; (v) Nanopore technology, another single molecule sequencing approach was in late-phase testing at the time of writing. These technologies, especially those that depend on nanopores and PacBio, are all in rapid development so no exhaustive review will be made here as it is likely to become outdated almost immediately. Of note is that the Roche 454 system is currently already out of production. Compared to 'Sanger' sequencing all of the current methods generate individual sequence reads with high error rates and error correction is achieved with very high sequence coverage.

The discriminatory capability of WGS is very high as it samples the whole genome, including extra-chromosomal DNA. Reproducibility and repeatability are also high. Current international harmonisation is lacking except for the availability of data management tools and annotation guidelines – however the latter does not provide a fully harmonised nomenclature. The potential for future international harmonisation is currently uncertain, but should be considered high from a technical point of view.

VIII – Evaluation for use in different public health-related applications

With regard to the review of the appropriateness of use of the different food-borne pathogen sub-typing methodologies for different food-safety related public health applications (i.e. detection and investigation of food-borne outbreaks of disease, food-borne source-attribution, early identification of food-borne organism with epidemic potential and their integration in risk assessment), it is concluded that detection of outbreaks and their investigation in real-time would be enhanced by the generation of fully comparable molecular typing data from human, veterinary and food laboratories prior to submission to a central or connected databases. Some molecular typing methods (e.g. MLST, PFGE, MLVA) have been harmonised to a greater or lesser extent for the purpose of outbreak detection and investigation. The international development of harmonised platforms for WGS-generated data should be encouraged.

In relation to source-attribution analysis of food-borne pathogens, the Panel concluded that a major challenge of using data generated from molecular typing methods in source attribution models, in particular WGS data, will be to define meaningful subtypes providing an appropriate level of discrimination for source attribution. A high level of discrimination is not necessarily the best option. The applied method has to allow for some genetic diversity between isolates from human and animal/food sources, but only to the degree so that it can still be assumed that they originate from the same source. Independent of the choice of molecular typing method and approach for source attribution, it is important that the data included from human and potential sources are related in time and space. Source attribution analysis is, therefore, facilitated by integrated surveillance providing a collection of isolates from all (major) sources that should, to the extent possible, represent what the human population is exposed to.

The epidemic potential of a food-borne strain within a bacterial species, or even within a subtype varies considerably, and is a function of its inherent genetic characteristics and their expression combined with ecological factors including the opportunities to spread in the food chain. Prediction of the public health risk and epidemic potential of emerging strains of food-borne pathogens has not yet been possible. Nevertheless, if an epidemic strain has already emerged in a certain region such a strain can be rapidly characterised employing current molecular typing methods and thus serve to identify the occurrence of such strains in other regions for risk management purposes. High throughput WGS technologies offer new opportunities to characterise bacterial strains in great detail. The genetic information that these technologies provide will, however, need to be considered together with gene expression, host and ecological factors, including the opportunities to spread in the food chain. Finally, although there are differences between bacterial species, the principle of assessing the gene content in relation to fitness as a means to assess risk potential that has been used for the four organisms considered in this opinion should be applicable to any bacteria.

Eventually, in the document a series of recommendations are made on important issues to be considered as these methods, in particular WGS analysis, have limitations when using the data they generate. Thus, modern molecular typing methods provide many opportunities for rapid and accurate determination of the genealogical relationships among bacterial isolates. Interpretation of the results generated by these methods for different public health applications requires this information to be placed in the context of the diversity, degree of genetic change (e.g. during storage of isolates or mutation during an outbreak and in reservoirs) and population structure of the particular pathogen in question. Therefore, large scale carefully co-ordinated studies are required to fully elucidate this. The development of more informative and easier to use bioinformatic tools for analysis of WGS data is needed. Multidisciplinary and integrated research programs are needed to develop and validate the use of detailed genetic information for 'predictive' hazard identification, accounting for gene expression and how this affects the fate of pathogens in the food chain and their interaction with human and animal hosts. Further recommendations are made on particular issues to aid the use of these methods and the data they generate for the different applications considered.

IX – Conclusions

The Biohaz Panel concluded that molecular typing methods should ideally provide appropriate discriminatory power, reproducibility, capability for international harmonisation and reduced handling of and exposure to pathogens in the laboratory. No current typing method, whether phenotypic or molecular, complies with all these expectations. Several methods are often used in combination in order to obtain the resolution needed. The methods applied depend on the pathogen and on the application sought. These methods have proven track records of use and for some of them, notably MLST and PFGE, extensive databases of valuable typing data have been collected. Further, methods based on WGS are increasingly replacing the numerous different methodologies currently in use in human and veterinary reference laboratories, and the same methods can be used for all organisms. An essential precondition is the availability of quality control methods, to ensure the reliability and consistency of molecular data generated, coupled with high quality bioinformatics support for the analysis of the data generated. Regarding WGS, limited knowledge is available in relation to the technical errors that occur during sequencing and analysis and on the effect of genetic drift in the different bacterial populations over time, which may complicate the interpretation of results. The international development of harmonised platforms for WGS-generated data and suitable databases that can link strain and epidemiological data whilst still allowing for confidentiality of personal of commercially sensitive information should be encouraged

References

ECDC (European Centre for Disease Prevention and Control), 2007. Surveillance of communicable diseases in the European Union. A long-term strategy, 2008-2013. Available online: http://www.ecdc.europa.eu/en/aboutus/key%20documents/08-13_kd_surveillance_of_cd.pdf.

ECDC (European Centre for Disease Prevention and Control), 2013. Surveillance of communicable diseases in Europe - A concept to integrate molecular typing data into EU-level surveillance. Version 2.4. 7 September 2011. Available online at: http://www.ecdc.europa.eu/en/publications/Publications/surveillance-concept-molecular%20typing-sept2011.pdf (last accessed on 13/12/2013).

EFSA (European Food Safety Authority), 2009. Report on the availability of molecular typing methods for *Salmonella*, *Campylobacter*, verotoxigenic *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* isolates from food, animals and feeding stuffs in European Union Member States (and in some other reporting countries). In: *The EFSA Journal*, 2009, 272r, pp. 1-52.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the evaluation of molecular typing methods for major food-borne microbiological hazards and their use for attribution modelling, outbreak investigation and scanning surveillance: Part 1 (evaluation of methods and applications). In: *EFSA Journal,* 2013; 11(12): 3502, 84 pp. doi:10.2903/j.efsa.2013.3502

Hallin M., Deplano A. and Struelens M.J., 2012. Molecular typing of bacterial pathogens: a tool for the epidemiological study and control of infectious diseases. In: Morand S., Beaudeau F. and Cabaret J. (eds), *New Frontiers of Molecular Epidemiology of Infectious diseases*. Springer: London.

Maiden MC, 2006. Multilocus sequence typing of bacteria. In: *Annu. Rev. Microbiol.*, 60, pp. 561-588.

Meinersmann R.J., Helsel L.O., Fields P.I. and Hiett K.L., 1997. Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. In: *J. Clin. Microbiol.*, 35, pp. 2810-2814.

Ribot E.M., Fair M.A., Gautom R., Cameron D.N., Hunter S.B., Swaminathan B. and Barrett T.J., 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. In: *Foodborne Pathog. Dis.*, 3, pp. 59-67.

Struelens M.J. and Brisse S., 2013. From molecular to genomic epidemiology: transforming surveillance and control of infectious diseases. In: *Euro. Surveill.*, 18, 20386.

van Belkum A., Tassios P.T., Dijkshoorn L., Haeggman S., Cookson B., Fry N.K., Fussing V., Green J., Feil E., Gerner-Smidt P., Brisse S. and Struelens M., 2007. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. In: *Clin. Microbiol. Infect.*, 13 Suppl 3, pp. 1-46.