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Next generation sequencing for non-typhoidal *Salmonella*: Characterization and identification of virulence and antimicrobial resistance for food safety surveillance - An overview

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Abstract- Next Generation Sequencing (NGS) technologies have revolutionized bacterium DNA analysis, providing a rapid and cost-effective tool for surveillance. NGS allows a variety of analyses, including multilocus sequence typing, identification of antibiotic resistance, and pathogenicity characterization. Non-Typhoidal *Salmonella* (NTS), a zoonotic pathogen, represents a significant threat to food safety since it is considered as a common contaminant of food animals. Genomic factors that encode bacterial virulence and antimicrobial resistance are crucial for understanding NTS pathogenesis. Next-generation sequencing enables molecular detection, serological assays, and genotyping, providing insights into virulence and antibiotic resistance factors. NGS coupled with bioinformatics tools, emerges as a potent molecular technology, transforming microbiology through genetic sequencing technologies. The landscape of molecular research in food safety is rapidly transitioning from traditional molecular subtyping methods to typing methods based on Next Generation Sequencing (NGS). In this review, we explore the use of next generation sequencing in the analysis of Non-Typhoidal *Salmonella* DNA, particularly its effectiveness in typing bacterial strains and identifying genes linked to virulence and antibiotic resistance. This highlights the significant contributions of NGS to microbiological research in food safety.

Key words: Antimicrobial Resistance gene, Multilocus Sequence Typing (MLST), Next-Generation Sequencing (NGS), Non-Typhoidal *Salmonella* (NTS), Serotyping, Virulence, Whole-Generation Sequencing (WGS)

INTRODUCTION

Non-typhoidal *Salmonella* (NTS) is a leading cause of food-borne diseases worldwide as it stands as one of the leading contributors to diarrheal illnesses, representing a major public health challenge. The genus *Salmonella* is prevalent among food animals, making it a common cause of foodborne diseases worldwide. Human infections caused by NTS primarily occur through the consumption of contaminated food or water.[1-3] Infections caused by the pathogenic bacterial species *Salmonella enterica* impose significant global health burdens. This widely distributed species comprises approximately 2600 distinct serovars categorized as either typhoidal or non-typhoidal *Salmonella*. Despite their genetic similarities, these two groups induce entirely different illnesses and elicit distinct immune responses in human beings.[4,5] Non-typhoidal

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Salmonella infections are considered zoonotic, as they can be transmitted between animals and humans. Salmonellosis encompasses a spectrum of illnesses affecting both humans and animals, ranging from acute gastroenteritis to bacteraemia and extra-intestinal infections. While NTS intestinal infections are typically self-limiting, prompt antimicrobial therapy is crucial when the bacteria disseminate outside the intestine.[6,7]

Salmonellosis is more prevalent in developing nations, with multidrug-resistant *Salmonella* posing a significant threat, particularly in South America, Africa, and Asia.[8] The inadequate control programs and the lack of information from developing countries contribute to the underreporting of *Salmonella* in global surveillance systems.[9] These countries, even though they are participating in the One Health program which addresses health issues in humans, animal, and environment, face challenges in its implementation. In these countries, particular attention is given to zoonotic pathogens like Non-typhoidal *Salmonella*. The latter illustrates the kind of zoonotic risk One Health strategies seek to address, especially where food safety and transmission of diseases from animals to humans are major public health concerns.[10,11]

The complexity of NTS is compounded by numerous genes encoding virulence and antibiotic resistance factors critical to the bacteria's pathogenesis.[12] *Salmonella* strains harbour numerous virulence factors distributed throughout the genome, including the *Salmonella* pathogenicity islands (SPIs) and mobile genetic elements like plasmids and prophages. Some components are shared across all *Salmonella* serotypes, while others are serovar-specific.[1] While the presence of various virulence factors within NTS underlines the complexity of their pathogenic mechanisms, the situation is further complicated by the emergence of antimicrobial resistance (AMR). The latter encompasses numerous genes encoding resistance to antimicrobial agents. These genes are often located on plasmids, transposons, gene cassettes, or variants of *Salmonella* Genomic Islands.[7]

To address *Salmonella*'s virulence and resistance challenges, Next Generation Sequencing (NGS) proves effective as an emerging technology allowing for the comprehensive analysis of an organism's genome. It proves to be a quick and efficient method in foodborne disease surveillance.[13] Compared to conventional testing methods, NGS provides more detailed information on the genotypic traits of a pathogen, including its virulence factors and antibiotic resistance determinants. This assists in understanding and controlling its pathogenic traits. Recent research indicates that NGS accurately predicts pathogenicity and antimicrobial properties of various pathogens, including *Salmonella*, providing valuable insights into the pathogenic potential of the bacteria.[1.14,15] Microbiology is thus undergoing a transformative revolution with advances in genome sequencing technologies. When combined with bioinformatics tools, next generation sequencing becomes a powerful technology with a wide range of applications.[16]

In this paper, we present a broad overview of Non-typhoidal *Salmonella*, addressing its taxonomy, nomenclature, pathogenicity and the critical issue of multidrug resistance. Additionally, we examine the role of Next Generation Sequencing (NGS) in revolutionizing NTS surveillance since these technologies enable a detailed analysis of pathogens' genomic traits, marking a transformative era in the field of food safety surveillance.

Non-typhoidal *Salmonella*: An overview

Global Impact of Non-typhoidal *Salmonella*

Salmonellosis is among the most prevalent infections worldwide. The global health burden caused by NTS is significant because these infections are being predominantly transmitted through the consumption of contaminated food or water.[17] In fact, 94% of *Salmonella* cases are linked to foodborne transmission, with common sources including raw vegetables, unpasteurized dairy products, undercooked eggs, poultry, beef, and pig.[18] NTS accounts for approximately 93.8 million cases of gastroenteritis annually worldwide, with an estimated 80.3 million cases attributed to foodborne transmission alone, and about 155.000 human deaths each year.[19,20] Salmonellosis encompasses a spectrum of illnesses ranging from self-limiting gastroenteritis to more serious, potentially fatal, extra-intestinal infections like bacteraemia. In fact, non-developing countries, particularly in Asia and Africa, are the most harmed with such pathogens.[21-28] In Africa, NTS infections, being one of the major causes of bacteraemia, appear to be endemic, mostly in children.[24,29] These illnesses most occur in sub-Saharan Africa. A recent meta-analysis showed that there were an estimated 535 000 non-typhoidal *Salmonella* invasive disease illnesses and that 77 500 deaths were due to this disease in 2017.[21,24-27,30] In Asia, similar death rates were observed for invasive NTS infections.[22,23] Overall, several Non-typhoidal *Salmonella*

serovars capable of causing invasive diseases are showing resistance to multiple antimicrobial agents, including those recommended for clinical treatments. The development of antimicrobial resistance in NTS serovars has far-reaching implications. Beyond compromising the effectiveness of standard treatment protocols, it significantly impacts clinical outcomes. Antimicrobial-resistant NTS infections often lead to more severe illness, prolonged hospitalization, increased treatment failure rates, and a higher risk of mortality.

History of *Salmonella*

Salmonella has a rich history dating back to the 1800s. In 1888, the successful cultivation of the organism was achieved by Salmon and Smith, while Gartner reported the initial human case and isolation of the bacteria.[31] The nomenclature "*Salmonella*" was established in 1900, a tribute to its discovery in the laboratory of Daniel Elmer Salmon.[32] Since then, significant confusion among strains has been observed in relation to the large number of isolated *Salmonella* strains. Only the detailed study of antigens has made possible the valid identification and classification of strains. The serological study describes the O and H antigens of *Salmonella*, enabling precise identification of the bacterium.[33] In 1926, Bruce White presented the first outline of the antigenic structure of *Salmonella* species. White's research was taken up and expanded by Kauffmann (1941), who developed the Kauffmann-White Scheme, the basis for all studies in the field of Salmonella. This scheme has gained general agreement from the scientific community. This scheme gives each known strain of *Salmonella* to date a distinct identity.[33-36]

The update of this scheme is the responsibility of the World Health Organization Collaborating Centre for Reference and Research on *Salmonella*, OMS-Salm (Pasteur Institute, Paris, France), in collaboration with the World Health Organization (WHO). New serotypes are continually documented in the list of the Kauffmann-White-Le Minor scheme,[35,36] last updated, to the best of our knowledge, in 2014.[36]

Bacteriological characteristics

Belonging to the Enterobacteriaceae family, *Salmonella* is characterized by consistent morphological and biochemical features. The bacilli typically measure between 2 to 5 µm in length and 0.7 to 1.5 µm in width. *Salmonella* is Gram-negative, oxidase-negative and the majority of its strains display motility, facilitated by peritrichous flagella, produces gas from glucose and exhibit the ability to reduce nitrates to nitrites.[37,31] In general, the biochemical characteristics of non-typhoidal *Salmonella* serotypes are as follows: lactose-, ONPG-, H2S+, LDC+, ODC+, urease-, indole-, gelatinase-, DNase-, Simmons citrate-, adonitol-, glycerol-, galacturonate-. Some phenotypic properties of *Salmonella* are so specific that they are used for the enrichment, selection, isolation, and differentiation of colonies. *Salmonella* and other Enterobacteriaceae are resistant to novobiocin, selenite, tergitol, and bile salts, especially deoxycholate. The most selective media such as the *Salmonella*-Shigella (S.S) medium, contains bile salts and brilliant green as selection agents, lactose and sodium thiosulfate as substrates, and neutral red and ferric citrates as indicators. *Salmonella* strains typically yield colonies (usually with a diameter of 2 to 4 mm) with a black center (indicating the presence of H2S). As a mesophilic bacterium, *Salmonella* grows at temperatures close to the body temperature of warm-blooded animals (35-43°C), with an optimal pH of 7.2.[38,37,31]

Taxonomy and nomenclature

The nomenclature of *Salmonella* is particularly complex as it has been the subject of controversies and confusion over time. Before 1973, Kauffmann proposed the idea that a serovar is equivalent to a species.[39,35] However, in 1973, DNA-DNA hybridization revealed the connection of all serovars at the species level, with *Salmonella bongori* as the exception.[40] The nomenclature shift led to recognizing two *Salmonella* species: *Salmonella bongori* and *Salmonella enterica*, with the latter having six subspecies (*enterica, salamae, arizonae, diarizonae, houtenae,* and *indica*).[36] In fact, Le Minor and Popoff's proposal avoided confusion between species and serovar names. They proposed not to consider the names of serovars as Latin names, to write them with an initial capital letter (for example, *Salmonella enterica* subsp. *enterica* serovar *enteritidis*). In common practice and to simplify the nomenclature, Le Minor and Popoff also proposed to designate serovars in an abbreviated form: *Salmonella enteritidis*.[41] The way *Salmonella serotypes* have been designated has evolved over time. Bacteriologists used to name them after the diseases they caused or the animal species from which the bacillus originated. This is how we have *Salmonella gallinarum, Salmonella abortusovis, Salmonella typhimurium*, etc. However, the designation of new serovars is based exclusively on the geographical origin.[35,42] Until the 2000s, 2463 serovars were identified, including 1454 belonging to the *enterica* species and 20 to

the *bongori* species. In 2014, the Kauffmann-White-Le Minor scheme recognized 2,659 serotypes, with 1,586 under the *enterica* species and 22 under *bongori*.[36]

Multi-drug resistant MDR *Salmonella*

A noticeable rise in multi-resistant strains has been observed, limiting treatment options and leading to heightened morbidity and mortality rates. In recent years, there have been concerning trends in the development of antimicrobial resistance among *Salmonella* strains, posing a significant public health challenge. From the 1950s onwards, the emergence of non-typhoidal *Salmonella* strains with decreased susceptibility to various antibiotic classes has been observed. The emergence of antimicrobial resistance is a common trend among bacterial pathogens that contaminate food animals, as these drugs are often used as growth promoters.[43,44] Indeed, the emergence of resistant bacteria can be attributed to the inappropriate use of antimicrobial agents especially in agriculture,[45] a concern that is particularly pronounced in developing countries.[46] It has often followed the use of multiple antibiotic classes in animals which contributes to the selection of resistant strains and their subsequent spread.[46,47] For example, the use of fluoroquinolones in chicken flocks can shift or replace bacteria from 100% susceptible to 100% resistant within a few days.[48] The use of third-generation cephalosporins can also be associated with the emergence of particularly concerning *Salmonella* strains due to their resistance to these antimicrobials.[49,50] The misuse or overuse of third-generation cephalosporins and fluoroquinolones in both human medicine and agriculture has led to the development of multidrug-resistant (MDR) *Salmonella* resisting not only cephalosporins and fluoroquinolones but also multiple other classes of antibiotics. These powerful antibiotics, while effective against a range of bacterial infections, can drive the selection of resistant strains among bacteria like *Salmonella*. Furthermore, ESBL (extended-spectrum Beta-Lactamase) producing strains, expressing resistance to the broad range of antibiotics; beta-lactams, are increasingly identified in *Salmonella* isolates.[51] NTS has also shown signs of acquired resistance to colistin, an antibiotic of last-line defence against several MDR bacteria.[52] Resistance to these potent drugs limits the available treatment options and can result in higher rates of treatment failure and complications.

To address these trends, there is a growing emphasis on *Salmonella* antimicrobial surveillance programs. Data provided by surveillance organizations show varying levels of bacterial resistance in different countries, due to differences in prescribing practices of antibiotics to animals destined for human consumption by farmers. However, due to the globalization of trade, multi-resistant strains are easily disseminated worldwide.[48] As a result of these dissemination patterns, it becomes crucial to understand how antibiotic resistance can emerge, either through mutations within bacterial populations or via the acquisition of resistance determinants through horizontal gene transfer mechanisms. The widespread use of antibiotics in natural habitats truly challenges the microbial populations in these ecosystems.[46,48] Research efforts are focused on understanding the genetic mechanisms underlying resistance and developing new strategies for combating drug-resistant bacteria.

Pathogenicity of *Salmonella* and virulence factors

Salmonella possess various virulence factors that allow the bacteria to adapt to environmental conditions and host responses at each stage of the infection process. Research has led to the discovery of unique protein secretion systems controlling the key steps of infection, such as invading epithelial cells and surviving within macrophages. *Salmonella* infections typically originate in the intestine. After colonization, the bacteria multiply in the digestive tract, with the cecum serving as a crucial site for their replication. After transient bacteremia, the bacteria could be found in regional lymph nodes and within the liver and spleen macrophages, organs that *Salmonella* have a particular affinity for. *Salmonella* can multiply within these organs before being disseminated through the bloodstream. In immunocompromised individuals, serotypes responsible for gastroenteritis in humans can enter the reticuloendothelial system and cause severe systemic disease. During gastroenteritis, the symptoms are partly related to the secretion of an enterotoxin but primarily due to the destruction of villi, disrupting absorption functions, and significant inflammation that increases secretions. In cases of septicemia, the endotoxin released from bacterial lysis is responsible for toxic shock.[53]

The outcome of a *Salmonella* infection largely depends on the virulence of the specific strain and the host's status. While factors like age, genetics, and the environment primarily determine the host's condition, the bacteria's characteristics are determined by virulence factors.[54] Several *Salmonella* virulence factors have been identified, including type III secretion systems, the Vi antigen, the lipopolysaccharides, the flagella, and various other factors

essential for the intracellular life cycle of the strain.[55] The genes encoding these factors are typically located on *Salmonella* Pathogenicity Islands (SPI).[55] SPIs are extensive regions of DNA inserted into the chromosome. They are defined as DNA segments encoding virulence genes. This DNA region is absent from the corresponding region in the genome of *E. coli* K12, which is known to be roughly colinear with the *Salmonella typhimurium* chromosome.[55] Key regions of SPIs are postulated to have played a major role in the divergence between *Salmonella* and *E. coli* over millions of years and appear to contribute to the adaptation of *Salmonella* to its hosts.[55] SPIs, of varying lengths, often carry large groups of genes contributing to a specific virulence phenotype.[56] The first pathogenicity island, SPI1, governs the ability of *Salmonella* to invade host epithelial cells through a type III secretion system. The second island, SPI2, controls the pathogen's survival in macrophages through a distinct type III secretion system. The major virulence factor of SPI3 is a system necessary for survival in the face of intramacrophage nutritional limitations.[57-59] The invasion function in *Salmonella* is not solely controlled by SPI1; genes from SPI4 also play a crucial role.[60] In general, more than 20 SPIs are identified in *Salmonella*, from which islands 1, 2, and 4 are potentially conserved within the genus, while the others are variable or partially variable.[59,61]

Next generation sequencing in *Salmonella*

The advent of Next Generation Sequencing technologies has revolutionized pathogen surveillance as rapid and efficient techniques enabling the comprehensive analysis of a bacterium's complete DNA sequence.[15] The transformative impact of NGS on enteric pathogen monitoring is now evident in public health laboratories, where it is replacing the reliance on multiple independent laboratory tests.[28] As the cost of these technologies is decreasing and run times are becoming more efficient and allows for the inference of a wide range of pathogen features in a single sequencing run, the techniques are expected to become more widely available in routine diagnostic laboratories.[28,62] In addition, recent applications in metagenome sequencing for infectious disease diagnosis and outbreak investigation demonstrate the potential for culture-independent pathogen detection from complex clinical samples The speed of sequencing, the direct extraction of serovar and multilocus sequence typing information from genome data facilitates the linkage of sequenced isolates to historical data, enabling the rapid tracing of the origin of contamination sources.[63,64] The primary analyses employed for these purposes include single-nucleotide polymorphism (SNP) analysis, based on the detection of variations in single nucleotide positions in the genome, core genome multilocus sequence typing (cgMLST), involving a gene-by-gene examination using only core genes, and whole-genome MLST (wgMLST), which encompasses MLST by using both core and accessory genes.[63-68]

Given the inconsistency of traditional molecular typing methods, Multilocus Sequence Typing (MLST) emerged as a more reliable and standardized approach for characterizing bacterial strains.[69] The MLST strategy aims to pinpoint internal nucleotide sequences of specific housekeeping genes, typically ranging from 400 to 500 base pairs. This approach assigns a unique "allelic profile," constituting a distinct set of alleles at each locus, to designate the sequence type (ST). MLST emerged when robotic sequencers made it possible to determine nucleotide sequences of a group of housekeeping genes, ribosomal genes, and/or virulence genes.[69,70] MLST has the capability to assess sequence changes at the level of a single nucleotide.[69] Since its inception, MLST, pioneered with the Neisseria scheme, has earned the status of the "gold standard" in typing. Subsequently, species-specific schemes for various bacteria and fungi have proliferated. The curated databases housing ST profiles and MLST allele sequences are globally dispersed.[71,72] Beyond its role in outbreak scenarios, MLST serves to categorize isolates across different species globally.[73,74] In diverse bacterial applications, the accurate and consistent classification of bacteria is paramount. Swift and precise identification of infectious agent strains is crucial, particularly during outbreaks.[73,74] When needed, MLST, alongside other genetic subtyping techniques, contributes a phylogenetic context within a serotype, a crucial facet in public health surveillance. The advent of cutting-edge technologies, such as Whole Genome Sequencing (WGS), facilitates the integration of identification, subtyping, and characterization workflows into a singular, comprehensive platform.[65]

The current era of high-throughput sequencing advocates for using Whole Genome Sequence (WGS) data for typing. The decreasing cost of DNA sequencing, technological advancements, and equipment cost savings render WGS affordable for individual researchers and routine laboratories. The higher quantity of complete MLST profiles obtained using WGS based approaches than with

the traditional method is proof of their superiority.[75] Multilocus Sequence Typing (MLST), which indicates to be an effective method for characterizing bacterial isolates, has been made more versatile by WGS strategies to accommodate high-throughput data.[76] The accessibility of WGS for routine laboratories opens avenues for trend research, diagnoses, and surveillance, promising quick completion times conducive to real-time surveillance and epidemic detection.[63] However, challenges lie in sifting through voluminous data and connecting WGS to typing schemes, enabling comparisons with other widely used technologies and historical data.

Next generation sequencing-related techniques are not only useful for identifying bacteria at different levels of classification but also allow studying genetic markers, such as those for virulence and antibiotic resistance. Understanding these markers is crucial for gaining insights into the pathogenesis of bacteria, the mechanisms behind resistance to antibiotics, and the epidemiology of infectious diseases.

Detection of virulence genes

Virulence factors enable bacteria to infect and cause illness in their hosts. These factors are crucial for bacterial infections, playing a key role in helping bacteria to evade or counteract the host's immune defense. They are vital for researching bacterial diseases and creating treatments.[77] Next-genome sequencing technologies have significantly advanced the studies on virulence markers in bacteria. Three main methodologies are used for the identification of virulence genes in bacterial genomes.[78-81] The first one is based on the comparison of genomes from strains with different virulence levels. It involves analyzing the genetic differences between highly virulent and less virulent strains. Tools like BLAST can be used for the alignment and the comparison of the genomes in order to identify differences in sequences associated to virulence. This method is commonly used in microbial genomics.[78,79,82] The second method involves the identification of laterally transferred genomic islands, and running the genome against databases of known virulence markers. This methodology considers virulence genes as being acquired via gene transfer. Software like Island Viewer integrates various genomic island prediction methods to identify potential horizontally acquired regions in genomes.[78] The third strategy proceeds with running the Genome against databases of Known virulence markers: This involves comparing a genome to existing databases to identify known virulence genes. Databases such as VFDB (Virulence Factor Database) are used in conjunction with tools like BLAST to identify known virulence genes in genomic sequences.[1,78]

Detection of resistance genes

Antimicrobial resistance poses a major worldwide health challenge, particularly in bacteria with decreased susceptibility to strong antibiotics like third-generation cephalosporins and carbapenems.[83] NGS allows the prediction of bacterial resistance types, even those not typically assessed through conventional antimicrobial tests. It enables real-time creation of antimicrobial resistance profiles and prediction of multi-resistant pathogens such as *Salmonella*.[28,65,84] In recent years, various methods and tools have been developed and published for detecting genetic factors responsible for antimicrobial resistance. These tools analyze data obtained from whole-genome sequencing (WGS) aiding in the comprehensive identification and understanding of resistance mechanisms.[85]

Generally, the methods used for finding resistance markers are similar to those used for detecting virulence genes. Three primary methods are available for identifying resistance genes. Genomic comparisons between resistant and susceptible strains are conducted using genomic alignments and comparisons tools such as BLAST. Resistance genes are also identified by comparing genomic sequences with established databases like CARD (Comprehensive Antibiotic Resistance Database), Resfinder, ARDB (Antibiotic Resistance Genes Database), ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) and MEGARes (MEtaGenome Analyzer for Antimicrobial Resistance) to identify known resistance genes in genomic sequences along with with the use of alignment tools.[28,86,87] The analysis of mutations conferring resistance with tools such as BWA, Samtools, or PointFinder also enables researchers to understand antimicrobial resistance mechanisms.[28,86,87] These emerging methods serve as valuable additions to conventional culture-based approaches in clinical and surveillance settings. They offer rapid and precise means to determine resistance in both cultivable and non-cultivable bacteria.[79,85]

Surveillance, Outbreaks & phylogenetic Investigations:

Next generation sequencing has proven to be an essential tool in epidemiology, enabling detailed analysis of infectious disease transmission. It has been particularly effective in tracking micro-epidemics and understanding the spread of diseases globally. Combined with

bioinformatics, it revolutionizing *Salmonella* outbreak surveillance. During infectious outbreaks, this approach enables precise and rapid identification of the involved strains. By comparing genomic sequences, researchers can trace infection sources, track disease spread, and establish connections among related cases. This tracing capability significantly enhances epidemiological investigations, enabling a swift response to contain disease dissemination. Moreover, this method establishes connections among bacterial strains from different geographical regions, aiding in understanding transmission patterns and anticipating risks of nationwide or even international spread. It plays a crucial role in managing public health emergencies and devising effective public health strategies to control *Salmonella* outbreaks. These technologies have enhanced outbreak detection by identifying outbreaks even with a smaller number of cases.[88-93] Moreover, it has significantly improved the ability to establish connections between human illnesses and specific infection sources. In this revelation, NGS presents opportunities to address inquiries regarding foodborne pathogens and potential preventive measures that were previously unattainable.[90] Whole genome sequencing WGS was first applied to investigate a severe listeriosis outbreak in Canada. The investigation involved two outbreak-related isolates with close PFGE patterns. WGS enabled a comprehensive genetic comparison, revealing distinct strains potentially contributing to the outbreak. Many studies showcased NGS technics as valuable tools applicable in urgent public health crises.[94,95] Since then, the consistent integration of NGS into disease surveillance has notably strengthened our capabilities in identifying and investigating outbreaks, as well as in tracking disease patterns.

The American national surveillance network for foodborne disease PulseNet, which controls subtyping of *Salmonella* and other bacteria, used to perform pulsed-field gel electrophoresis (PFGE) as a standard method for molecular subtyping, until 2015. Then, laboratories introduced WGS and collected sequences data to standardize genotypic serotyping by SeqSero (http://www.denglab.info/SeqSero). The PulseNet database is using core genome MLST (cgMLST) and Single-nucleotide polymorphism SNP analysis to exhibit the phylogenetic relationships of the bacterial isolates. Antimicrobial resistance is also studied by predicting antimicrobial resistance determinants using in silico tools such as ResFinder.[96]

In silico and Bioinformatics Tools:

The assembly of the *Salmonella* genome is a fundamental step involving reconstructing the complete genome sequence from raw sequencing data. In silico genome assembly from sequencing data process DNA fragments to accurately and exhaustively reconstruct the bacterial genome sequence.[97,98] This step is crucial for identifying specific genes, coding regions, and functional elements within the *Salmonella* genome. As for annotation, it represents the process of identifying and characterizing genes and functionalities within the assembled genome. Annotation tools automatically annotate genes based on their presumed function. These annotations are valuable for understanding the roles of genes in pathogenicity, drug resistance, and other biological characteristics of *Salmonella.*[99,100] The evolution of in silico tools in Whole Genome Sequencing (WGS) has seen significant progress driven by computational advancements. Early tools struggled with short-read sequencing but gradually improved with novel algorithms. With the rise of long-read sequencing, new tools, offering better handling of longer reads and more accurate assembly. Hybrid approaches combining short and long reads further enhanced accuracy and contiguity. Ongoing advancements prioritize speed, accuracy, and scalability, refining algorithms to handle large datasets efficiently while improving genome assembly's precision and completeness.[101,102] Specialized bioinformatics tools like ResFinder, CARD, VFDB, or Victors are designed to detect genes associated with antimicrobial resistance and virulence factors within *Salmonella* genomes. These tools use genetic data to predict potential virulence and antimicrobial resistance and identify the corresponding contributors and mechanisms. The latter provides crucial information to understand disease dynamics and adapt treatment strategies. By identifying resistance genes in *Salmonella* strains, these tools help anticipate resistance profiles and guide treatment choices, contributing to a more targeted and effective approach to combat infections.[103-105] The development of such tools makes food safety research shifting away from traditional molecular subtyping approaches toward NGS-based characterization methods.

CONCLUSION

In summary, Next generation sequencing technologies such as whole genome sequencing have revolutionized the field of microbiology, particularly in food safety and public

health. These technologies provide unparalleled insights into the genetic content of pathogens which enhances the understanding of disease transmission and tracking. As NGS evolves, it promises to become an even more integral tool in microbiological research and outbreak response, with potential applications expanding into more efficient, high-resolution typing for a wide range of bacterial strains. Overall, globally, food safety surveillance knows a transitional phase from traditional molecular subtyping to next-generation sequencing methods since it can identify rapidly and with high precision genotypes, virulence factors, antibiotic resistance, and phylogenetic relationships of the bacterial strains, replacing conventional tests, especially for hard-to-grow microorganisms. However, challenges related to the practical limitations of its implementation, like the need for sophisticated data interpretation, regular database updates, and advanced software persist, especially within developing countries.

AUTHOR CONTRIBUTIONS

All authors contributed to the concept of the study. Anubha Kumari- Conceptualization, writing-original draft Validation writing, review and editing; Amal Ben Hassena: Resources review Analysis, Software, Supervision, Validation, Visualization and editing; Anjali Kumari: validation of the work Software and review and editing of the draft; Avinash Kumar: Supervision, Validation writing, review and editing. All authors approved the final version submitted for publication and take responsibility for the statements made in the review.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA SHARING STATEMENTS

The review is based on the references cited. All data generated and analyzed during the study are included in this published article and the citations herein. Further details opinions and interpretation are available from the corresponding author on reasonable request.

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