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Review Article

Campylobacter Species, Microbiological Source Tracking and Risk Assessment of Bacterial pathogens

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Abstract

Campylobacter species continue to remain critical pathogens of public health interest. They are responsible for approximately 500 million cases of gastroenteritis per year worldwide. Infection occurs through the consumption of contaminated food and water. Microbial risk assessment and source tracking are crucial epidemiological strategies to monitor the outbreak of campylobacteriosis effectively. Various methods have been proposed for microbial source tracking and risk assessment, most of which rely on conventional microbiological techniques such as detecting fecal indicator organisms and other novel microbial source tracking methods, including library-dependent microbial source tracking and library-independent source tracking approaches. However, both the traditional and novel methods have their setbacks. For example, while the conventional techniques are associated with a poor correlation between indicator organism and pathogen presence, on the other hand, it is impractical to interpret qPCR-generated markers to establish the exact human health risks even though it can give information regarding the potential source and relative human risk. Therefore, this article provides up-to-date information on campylobacteriosis, various approaches for source attribution, and risk assessment of bacterial pathogens, including next-generation sequencing approaches such as shotgun metagenomics, which effectively answer the questions of potential pathogens are there and in what quantities.

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INTRODUCTION

Campylobacter spp cause campylobacteriosis, a chronic enteric infection. *Campylobacter* spp. are among the leading causes of gastroenteritis globally[1]. Importantly, World Health Organization (WHO) has identified *Campylobacter* species as one of the high-priority antimicrobial resistance. The evolution of antimicrobial resistance poses an additional threat to modern medical procedures, rendering current intervention measures geared towards curtailing the menace ineffective and increasing the mortality rate, causing treatment failure and infections—the spread of resistance genes through the environment[2]. Although the environment has been described as the reservoir of antibiotic-resistant bacteria which can be transmitted to humans, the environmental load of antibiotic-resistant *Campylobacter* is scarcely investigated[3]. Ingestion of contaminated water, as well as food, is the principal risk factor of campylobacteriosis[4].

There are various methods of source tracking and microbial risk assessment, most of which rely on conventional microbiological techniques. Detection of fecal indicator organisms such as *Escherichia coli* has been used as a traditional surface water pollution monitoring and risk assessment method[5]. However, this method is hampered by several limitations: poor correlation between indicator organism and pathogen presence and the inability of the method to indicate the source

of fecal pollution since indicator organisms are excreted by some warm-blooded animals, although source tracking is an essential tool for public health risk characterization and the subsequent implementation of remediation and control strategies[6-8]. In the last few years, novel microbial source tracking methods have emerged to mitigate these challenges. These include library-dependent microbial source tracking and library-independent source tracking. However, library-dependent microbial source tracking methods have several setbacks, such as poor interspecies sensitivity, specificity, and overall accuracy[9]. Interestingly, library-independent techniques such as quantitative PCR (qPCR) have allowed the accurate study of fecal pollutants in environmental samples, including water, by quantifying the host-specific microbial source by tracking gene markers[10]. In the library, independent techniques, *Bacteroidales*, as bacteria with a strict requirement for the absence of oxygen inhabiting the human and animal gut with a higher population relative to *E. coli*, are typically used as the target[11]. Host-specific *Bacteroidales* 16S rRNA gene markers have been developed for diverse hosts to segregate human and non-human fecal sources in the environment[12]. However, instead of targeting *Bacteroidales* 16S rRNA, a recent study reports that bird feces could be discriminated from other fecal sources by targeting bacterial taxonomic groups like species of *Helicobacter* with better results[13]. Again, these methods are not without limitations. For example, studies have reported that variations in geographical locations could seriously interfere with the performance and results of these microbial source tracking techniques[14,15]. Equally, it is impractical to interpret qPCR-generated molecular markers to establish the exact human health risks even though it can give information regarding the potential source and relative human risk[16]. Other techniques of microbial source tracing depend on the results of antibiotics resistance and carbon utilization assays[17]. In light of the limitations of these methods, it is, therefore, necessary to look inward to find alternative options that are robust in terms of sensitivity and specificity.

Recent advances in next-generation sequencing (NGS) approaches (**Figure 1**), such as shotgun metagenomic sequencing, have resulted in its widespread application in every aspect of microbiology, microbial source tracking inclusive[18]. Shotgun metagenomics can effectively answer the questions of what potential pathogens are there in a sample by identifying virulence and resistance genes and in what quantities[19]. When analyzed using an appropriate source tracking algorism, shotgun metagenomics data becomes a powerful tool for microbial source tracking and risk assessment[20].

Shotgun metagenomics is widely applied in environmental and clinical studies[21]. Metagenomics sequencing has been used to systematically study antibiotic genes associated with the human microbiome[22], study the links of the microbiome with inflammatory bowel diseases[23], and, importantly, track outbreaks of human pathogens[24]. Therefore, we set out to provide information on various source attribution methods and risk assessment of bacterial pathogens, highlighting the potential of next-generation sequencing in combination with machine learning technology.

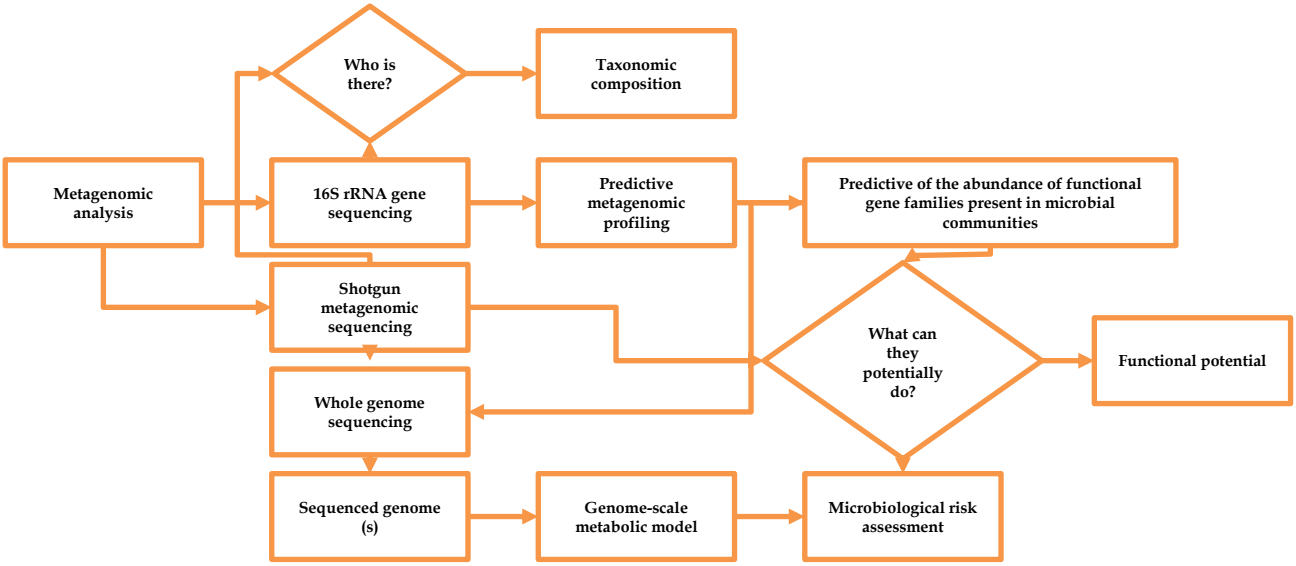

Figure 1. The links between metagenomics and microbial risk assessment[25]

MEDICALLY IMPORTANT *Campylobacter* spp., RESISTANCE GENES, AND RESERVOIRS

Campylobacter species, gram-negative, slender, spirally curled, and microaerophilic bacteria are essential etiologic agents of gastroenteritis in humans, responsible for approximately 500 million cases of gastroenteritis per year globally[1]. Veron and Chatelain[26] were the first to carry out a broad taxonomic study on the *Campylobacter* genus and classified them into four different species: *C. fetus, C. coli, C. jejuni*, and *C. sputorum* nearly five decades ago. Ever since, at least 36 species and 14 subspecies of *Campylobacter* have been described[27]. These include *C. upsaliensis, C. ureolyticus, C. helveticus, C. rectus, C. showae, C. gracilis, C. hominis, C. curvus, C. concisus, C. insulaenigrae, C. hyointestinalis*, and *C. lanienae*. Of all these, *C. jejuni* and *C. coli* are considered to be the leading cause of human campylobacteriosis[27,28]. Various extra-gastrointestinal conditions and autoimmune diseases, especially Guillain–Barre syndrome, have been mainly linked to *C. jejuni*[27]. However, pathogenicity in other species such as *C. lari, C. fetus, C. ureolyticus, C. upsaliensis, C. hyointestinalis*, and *C. concisus* has been documented[29,30]. Species of *C. fetus* have been isolated in septicemia patients and are frequently described as the etiologic agent of poor fertility and miscarriage in humans and animals[27]. It is therefore clear that the accurate tracking of these pathogens is crucial given their wide-ranging medical significance, especially as a number of them have been identified to harbor antibiotic resistance genes (**Table I**).

Table I. Medically important *Campylobacter* spp., resistance genes, and reservoirs

Campylobacter spp.	Resistance genes	Primary reservoir	References
C. jejuni	CmeDEF, *erm*(B), *aadE*, *sat4*, *aphA-3*, *tet*(O), *ant-like* A, *ant-like* B, *ant(6)-Ia*, *sat-1*, *sat-4*, *lnuC*, *ant(6)-Ib*, *aad9*, *aph(3)-IIIa*, *aph(2)-IIIa*, *hpt*, *apmA*, bla_{OXA-61}, *gyr*A and CmeABC	Dogs and cats	27,31-35
C. coli	*erm*(B), CmeABC, *aadE*, *sat4*, *aphA-3*, *tet*(O), bla_{OXA-61}, *cat*, *cfr*(C), *gyr*A, *ant-like* A, *ant-like* B, *ant(6)-Ia*, *sat-1*, *sat-4*, *lnuC*, *ant(6)-Ib*, *aad9*, *aph(3)-IIIa*, *aph(2)-IIIa*, *hpt*, *apmA* and *lnu*Cs	Dogs, cats, pigs and poultry	27,31,33-37
C. upsaliensis	*tet*(O) and *gyr*A	Dogs and cats	38,39
C. fetus subsp. *fetus*	*gyr*A, *tet*(44) and *ant(6)-Ib*	Cattle and sheep	40,41
C. rectus	*erm*(B)	Human oral cavity and dogs	42-44
C. hyointestinalis	*gyr*A	Cattle, pig and sheep	44,45

Campylobacter jejuni and *C. coli* exhibit intrinsic resistance to bacitracin, novobiocin, penicillin, rifampicin, trimethoprim, sulfamethoxazole, vancomycin, and most of the cephalosporins, whereas resistance to aminoglycosides, quinolones, macrolides, ketolides, amphenicols, and tetracyclines is usually acquired[46-48]. Although macrolides, such as azithromycin, and fluoroquinolone, such as ciprofloxacin, are the primary and secondary drugs of choice for the treatment of campylobacteriosis, resistance to these important antibiotics among species of *Campylobacter* with the potential to bring about more severe consequences, including prolonging hospitalization and higher risk of invasive infection or even death, have been reported[27]. This is of enormous concern, particularly when the global public health experts are struggling to contain the menace of antimicrobial resistance. What is more concerning, though, is that various mechanisms of resistance and, in some cases, a combination of more than one mechanism have been identified in these pathogens[49]. **Table II** summarizes the various mechanisms of resistance identified in *Campylobacter* spp.

MICROBIAL RISK ASSESSMENT

Quantitative microbial risk assessment modeling has been used to evaluate the risk of disease from waterborne pathogens since the 1980s. It is a type of modeling used to outline the human risk of exposure to disease-causing microbes from the environment through a dose-response model[50]. These models consist of several probability steps that rely on literature or primary data. Before the 2010 Haiti cholera epidemic, only a few studies, such as an analysis of the 1993 cryptosporidium outbreak in Milwaukee, Wisconsin, and an analysis of epidemic and endemic conditions caused by waterborne pathogens, applied mathematical modeling to study the transmission of the etiologic agents[51,52]. However, the Haiti cholera epidemic

shattered the country in 2010 and triggered a significant interest in applying infectious disease transmission modeling methods for waterborne microbial risk assessment; ever since significant progress has been made[53].

Table II. *Campylobacter* spp. mechanisms of resistance to various classes of antibiotics

Class of antibiotic	Mechanism of antibiotic resistance	References
Aminoglycosides such as gentamicin, amikacin, tobramycin, neomycin, and streptomycin.	a). Enzymatic modification and inactivation of antibiotics	[27,54]
Macrolides, lincosamides and ketolides. Examples include erythromycin, roxithromycin, azithromycin and clarithromycin.	a). Target mutation in 23S rRNA or/and ribosomal proteins L4 and L22 b). Modification of the ribosomal target by methylation through *erm*(B) c). Multidrug efflux pump (CmeABC) and altered membrane permeability	[55,56]
Quinolones such as levofloxacin (Levaquin), ciprofloxacin (Cipro), ciprofloxacin extended-release tablets, moxifloxacin (Avelox), ofloxacin, gemifloxacin (Factive) and delafloxacin (Baxdela)	a). Modification of DNA gyrase target (Thr86Ile) b). Multidrug efflux pump (CmeABC)	[27,57]
Tetracyclines such as tetracycline, doxycycline, minocycline and tigecycline	a). Protection of the ribosomal binding site by ribosomal protection proteins (RPPs) encoded by *tet*(O) b). Multidrug efflux pump (CmeABC)	[58,59]
β-Lactam antibiotics (penicillins and cephalosporins) such as carbenicillin, penicillin G, ticarcillin, ampicillin, nafcillin, cloxacillin, mezlocillin, oxacillin, and piperacillin.	a). Enzymatic inactivation of the antimicrobials by β-lactamase (OXA-61) b). Multidrug efflux pump (CmeABC)	[27,60]

Dose-response models are response curves produced by plotting the probability of a response outcome such as infection, illness, or death versus the known dose of the etiologic agent via an identified transmission route. Dose-response model is the main component of quantitative microbial risk assessment[61]. It is so crucial that a complete quantitative microbial risk assessment model is almost impossible to develop without it. Dose-response modeling can be regarded as a multidisciplinary area requiring substantial knowledge and skills in microbiology, pathology, mathematics, statistics, and computing[62]. In order to understand the procedure employed in the development and delivery of inoculum, as well as assess the employability of the data to the dose-response model, microbiology skills are necessary[63].

On the other hand, knowledge of pathology is required to assess the relevance and setbacks of identified exposure routes. To understand how to develop approaches to improve a model and write the required code to run such algorithms, computing and mathematics skills are needed. Furthermore, statistics knowledge is necessary for determining the confidence associated with employing the dose-response model across multiple hosts, pathogen strains, pathogen isolates, and routes of exposure[64]. In the design of a dose-response model, dosing experiments are typically carried out on animal models. Here a fixed concentration of pathogens is introduced to animals, and the resulting response is observed. The outcomes obtained are then incorporated into exponential or β-Poisson models, which will produce numerical constants that would calculate the probability of response outcome possible. Pathogen's concentration needed to trigger a response in ½ of the tested population would be regarded as either lethal dose-50 (LD_{50}) or infectious dose-50 (ID_{50})[61].

The dose-response models currently available in quantitative microbial risk assessment software packages are fixed, based on the pathogen(s) chosen or sole pathogen(s). The packages do not make it possible for researchers to choose a dose-response model or learn more about dose-response modeling in general, hindering users' ability to visualize and optimize the dose-response model[65]. For example, QMRASpot, a quantitative microbial risk assessment software developed by Kiwa Watercycle Research (KWR) which precisely models drinking water systems for the Dutch government, has its overall exposure pathway and dose-response models embedded, unchangeable, and cannot be independently visualized[66]. Similarly, The FDA-iRISK, an integrative comparative risk assessment system primarily designed for food-borne hazards, displays the dose-response model name and its functional forms. It also updates the dose-response model regularly using expert elicitation from dose-response experts, but the capability to choose, optimize or visualize the dose-response models is unavailable[67]. Noteworthy, dose-response models for many infectious bacteria, including antibiotic-resistant bacteria, are lacking, and whether dose-response between antibiotic-resistant and susceptible bacteria might vary remains unknown. Therefore, to bypass these limitations associated with dose-response models[61].

APPLICATION OF SHOTGUN METAGENOMICS AND MATHEMATICAL MODELS IN RISK ASSESSMENT

Application of high-throughput sequencing techniques such as shotgun metagenomics can allow genomic analyses and identification of genes present in genomes of all microbial communities and the protein in a sample without the need for prior culture in the laboratory[68]. Shotgun metagenomic sequencing is a type of sequencing that reads out the nucleotide bases of all microbial DNA present in a sample without targeting a particular genomic locus[69]. Here, microbial DNA is typically extracted and pruned into small chunks sequenced severally rather than targeting a specific genomic locus. This will produce DNA reads that align to distinct genomic locations for the various genomes present in the sample. This approach allows resistance and virulence genes to be identified, cloned, and functionally expressed[70]. In comparison to 16S rRNA gene amplicon sequencing, which only profiles targeted organisms or particular genes, shotgun metagenomics sequencing has been proven to provide results with enhanced resolution, better sensitivity, and more broad characterization of microbial communities in samples. This has led to its widespread application across the globe in various fields of scientific research[71].

Since its introduction almost two decades ago, metagenomics approaches have been applied to various studies, including characterizing endosymbiotic bacteria from the environment, identification of bacterial species capable of carrying out total ammonia nitrification, detecting of presence of antibiotic-resistant genes in bacteria from the gut, investigating human pathogen outbreak and study of diversity and function of microorganisms living in different types of water samples[72]. Specifically, shotgun metagenomics has been employed to characterize taxonomic and functional shifts in hot water microbiomes and established that unassembled short metagenomic reads were efficient for broadly screening for the potential presence and quantities of pathogens of interest in water[73]. Likewise, in a recent study, Chen *et al.*[74] carried outsource the identification of antibiotic resistance genes of an interconnected river-lake system using shotgun metagenomics and observed an abundance of assorted genes linked to sewage pollution from city effluents. Further, in a different study[75], a shotgun metagenomic study brings sand from freshwater beaches as a source of disease-causing bacteria. Hence, the exploitation of this approach in microbial risk assessment no doubt offers significant potential in discovering resistance and virulence genes among members of *Campylobacter* in the water system.

Targeted screening method using the 16S rRNA gene marker for bacteria and shotgun metagenomics approach, which allows for the broad-range simultaneous detection of all microorganisms using the complete genetic information in the sample, are the two classical approaches commonly employed to study the composition of metagenomics samples[76]. The 16S rRNA gene, found in the genetic material of every bacterium, has alternating and conserved regions. The conserved areas of the 16S rRNA gene allow for amplifying the nine variable regions using specific short single-strands of nucleic acid called primers. The amplification products are then processed for sequencing in a library construction process[77]. Typically, shotgun metagenomics constitutes six steps from study design to data validation. There is sample collection; processing and sequencing; pre-processing of the sequencing reads; profiling of taxonomic, functional, and genomic features; and data analysis[78]. Every stage of this multi-sequential requires careful preparation and excursion, especially since every step has several pitfalls that can affect the final result. To ensure the lysis reagent has access to the nucleic acid, adequate homogenization, and cell lysis before nucleic acid extraction must be achieved[79].

Phylogenetic analyses of pathogenic microbes using next-generation sequencing approaches like shotgun metagenomics are potent tools for tracking the origin of disease, examining the evolutionary relationships, and deciphering the transmission pathways[69]. Shotgun metagenomics is so robust that it can be employed in taxonomic characterization and understanding the relationships between microorganisms, their activities, and functionalities in a given environment. This way, interest can be in the presence of antibiotic resistance and virulence genes and their transcripts[80]. Using an appropriate bioinformatics analysis tool or microbial risk assessment model, data generated from shotgun metagenomics can be analyzed to investigate an outbreak, source attribution, and risk assessment, depending on the study's objectives. Therefore, the potential this kind of powerful approach holds cannot be overlooked[81].

APPLICATION OF WHOLE GENOME SEQUENCING AND METAGENOMICS IN OUTBREAK INVESTIGATION, SOURCE ATTRIBUTION AND RISK ASSESSMENT OF FOODBORNE PATHOGENS

Whole genome sequencing (WGS) and metagenomics are powerful tools in contemporary food safety studies because they make possible robust and timely detection, identification, and characterization of a wide range of foodborne pathogens. During an outbreak, a credible, rapid and powerful identification technique is invaluable in curtailing the etiologic agent's further spread and avoiding false source attribution[82]. Either culture-based or targeted techniques commonly identify foodborne pathogens. Targeted identification techniques such as PCR or ELISA, although rapid since they can be carried out without the need for prior culture, are not potent and therefore allow unrepresentative strains to go undetected. In addition, because of their low molecular level resolution, these techniques are incapable of establishing the link between an outbreak and detected pathogenic microorganisms[83].

In recent years, the development of novel source-tracking models has been rapidly triggered by a surge in the application of WGS in food safety and public health. Various models and machine learning algorithms have now replaced conventional risk assessment models. Bioinformatics data sharing tools make it particularly crucial as it allows efficient use of WGS and metagenomics in risk assessment, source tracking, and outbreak investigations, specifically at local, regional, national, and international levels[84]. Whole genome sequencing is a powerful molecular technique with a high ability to discriminate among isolates. Thus, it can be employed to establish the relationship between an outbreak and a specific pathogen. Although its laborious nature has limited its application to research settings rather than routine food screening, quite several researchers have successfully employed WGS for source tracking in retro-perspective studies of enterohemorrhagic *E. coli*[85], *Salmonella* Bareilly strain causing a foodborne outbreak[86], and protracted invasive listeriosis Outbreak in Germany[87].

Further, the use of WGS in outbreak investigation in the food industry by the United States Food and Drug Administration and the Centre for Disease Control is increasing. For the outbreak investigation, data generated from WGS studies are deposited to the GenomeTrakr, an open-access database. Currently, the GenomeTrakr database consists of laboratories in the US and worldwide, resulting in a significant data increase[88]. GenomeTrakr and similar databases employed in outbreak investigations are making it increasingly possible to decipher the links between sequence data from disease outbreaks on the one hand and food and environmental sources on the other. Similarly, the capacity of WGS to discriminate isolates based on their sources makes it possible to detect diffuse outbreaks by linking rare cases, which would ordinarily be regarded as sporadic cases lacking a common source. This will go a long way in mitigating disease outbreaks from their source[89].

Phylogenetic data can be employed in source attribution since source attribution aims to measure the corresponding significance of particular food sources and animal reservoirs for human cases of foodborne diseases. The genetic information could indicate possible relationships with specific hosts or reservoirs and therefore provide hints on a particular foodborne path's geographical distribution and transmission path[90]. In identifying transmission routes by determining the epidemiological links between reservoirs or sources of infections and supplanting the epidemiological data, WGS is an efficient technique. This approach has proven efficient for several foodborne pathogens such as *Salmonella*, replacing traditional source tracking methods, which are often insufficient and inaccurately attribute the source of contamination[91].

Metagenomics, as a technique that does not rely on a prior culture of samples, has the potential to contribute significantly to outbreak investigation, and risk assessment in food microbiology, particularly as it relates to the detection and characterization of non-culturable, fastidious microbes, the source attribution of risk related to virulence and resistance genes, as well as assessment of microbial risk in complex communities[82].

The application of metagenomics sequencing makes it possible for the synchronous detection and identification of the etiologic agent, antimicrobial resistance, and virulence genes, providing potential as a reliable technique for examining food and water quality[92]. The application of metagenomics in food safety to detect pathogenic microorganisms in foods is one major area that has received attention in recent years. In addition to detection and identification, analysis such as source attribution and risk quantification might be desired. The pathogenicity of some food pathogens, such as the *Bacillus cereus*,

which have very similar genomes, can be determined using virulence determinants encoded on their extrachromosomal DNA. The combination of data such as the presence of pathogens and specific virulence markers is necessary for risk assessment associated with these bacteria in contaminated food[82]. In order to detect foodborne pathogens using metagenomics, the application of shotgun sequencing has been recommended since it allows the detection and characterization of microorganisms from various forms of samples[93].

Using the metagenomics approach, detection of disease-causing bacteria involves taxonomic profiling of shotgun sequencing data using bioinformatics tools which could produce false results, especially at the species level. This could bring about the detection of less pathogenic or opportunistic pathogens rather than human pathogens, leading to underestimation or overestimation of the potential risk. It is, therefore, necessary to verify results[94]. Moreover, species-level identification is inadequate in assessing the potential risk of foodborne pathogens. Thus, it is necessary to determine virulence and resistance genes[95]. One other problem of taxonomic classification using metagenomics in risk assessment is that it detects hundreds of species of organisms, including those not of health significance, in a sample. Therefore, to detect species pertinent to risk assessment, it is indispensable to target pathogens, thence effortlessly removing trivial data for risk assessment. Doing this will no doubt minimize one of the major challenges of metagenomic studies, the difficulties associated with data analysis[96].

For risk assessment in food samples using metagenomic analysis, Grützke *et al*.[97] proposed a workflow in which the first identification of taxonomic units with kraken2 using the complete RefSeq database. Then from the list of species, human, animal, or plant pathogens are filtered, classified reads are extracted from the metagenomic dataset and verified with BLAST using the nucleotide database from the website of the National Center for Biotechnology Information (NCBI). Subspecies are resolved by determination of the closest available reference using Mash. Virulence factors are detected with SRST2 in combination with the Virulence Factor Database (VFDB). Metagenomics, especially when integrated into predictive models, has made a significant contribution to risk assessment investigations since it can answer questions related to risk assessment, such as what pathogens are found in food and how they interact as well as how environmental factors affect features of the foodborne pathogens such as virulence and resistance[82].

Despite its potential and numerous advantages, metagenomics sequencing has hurdles surrounding its applicability, efficiency, cost, and standardization. Shotgun sequencing, for example, is incapable of discriminating between viable and dead organisms. Interestingly, several wet-lab scientists and bioinformaticians are increasingly providing solutions to these challenges[98]. To assess the potential infection risk posed by *Campylobacter*, it is necessary to employ techniques that ascertain viability since the viability of pathogens is an essential parameter in food and water quality assessment[99]. Conventional culture-based techniques, which rely on the ability of viable microbes to take up nutrients and produce colonies in a culture medium, have been used for many years, but these methods are both arduous and time-consuming[100]. For example, *Campylobacter* spp. take a week or more to produce a positive detection result using the culture method. In addition, the sensitivity of culture methods is low since they are not always capable of detecting microbes in viable but nonculturable states, even though their detection is necessary to prevent disease outbreaks[101].

Various novel viability assays such as dye-based assays, phage-based assays, testing of cellular metabolism as well as the measurement of heat flow and ATP production have emerged in the last twenty years[102]. Viability PCR or vPCR has been widely employed, reviewed, and optimized as an efficient method for discriminating viable from inactivated cells. The underlying principle of vPCR is that it correlates viability with cell envelope permeability. Here, microorganisms in a sample are incubated with a dye such as a propidium monoazide (PMA). Following photo-activation, dye binds to exposed DNA and interferes with the amplification during PCR. Inactivated or dead cells with damaged membranes have their nucleic acids exposed to the dye. Once the dye-DNA complex is photo-activated, the amplification of non-viable cells is blocked[103]. On the contrary, viable cells having their cell membranes still intact exclude the dye, leading to strong quantitative PCR (qPCR) signals in the presence of the dye[104]. Viability PCR has been employed to study the viability of not just commonly studied bacteria but also fastidious bacteria, spore-forming bacteria, protozoans, fungi, and even viruses. This aggrandizes how efficient the technique is in distinguishing dead microbial cells from viable cells and how useful it can be in microbial risk assessment[105].

INFECTIOUS DISEASE TRANSMISSION AND QUANTITATIVE MICROBIAL RISK ASSESSMENT MODELING

Both infectious disease transmission and quantitative microbial risk assessment modeling have been employed to decipher the source and degree of infectious disease risk, the role of various routes of transmission as well as possible control strategies[106]. Infectious disease transmission modeling has been used for decades by infectious disease epidemiologists to carry out epidemiological studies. One such modeling framework is the susceptible–infectious–recovered, which models person-to-person contact and infection transmission in a given population and has been in use since the 1900s[107].

Infectious disease transmission models use mathematical equations to visualize the spread of pathogens within a population. They can be used to determine the direction and degree of disease outbreaks and generate information on factors that influence disease transmission and the impact of the containment strategy[108]. In infectious disease models, it is usually assumed that individuals infected with an infectious disease are capable of spreading the disease to other individuals in the population[109]. In order to understand this process of transmission, infectious disease transmission models use various variables representing the numbers of individuals of several different attributes associated with infection in a population. Typically, these attributes include susceptible, exposed, infected, and removed. In the infectious disease transmission modeling, whether an individual is regarded as infectious or otherwise must be considered[110]. In a population, those who are infectious are those who are infected and could potentially spread infectious agents to other individuals. In contrast, those who are not infected but can acquire the infection are regarded as susceptible individuals within the population[106].

On the other hand, individuals who associate with the infected individuals who might have been infected but are not yet infectious are regarded as exposed, and lastly, those who have recovered and are no longer infectious and are immune from re-infection are referred to as removed. Being removed may mean such an individual was killed by the infection or developed complete post-recovery immunity. The underlying point is that removed individuals are incapable of further transmitting the infection[111]. Mathematical models that rely on the susceptible, infectious and removed attributes are referred to as the Susceptible-Infectious-Removed (SIR) models. In SIR model, the flow of infection typically starts from susceptible to removal. Individuals usually start as susceptible, become infective at a given time, recover after a certain infectious period, and thence become removed. This way, the possibility of acquiring infection for a susceptible individual usually relies on the status of individuals in the SIR model, which is the leading principle for the classic non-linear dynamics within disease transmission. On the other hand, the timing of removal following infection (the infectious duration) typically does not depend on other individuals and their status[112].

A simple SIR model can be expanded to include additional attributes germane to the transmission dynamics of a particular disease of interest. The attribute 'exposed' is often included, resulting in a corresponding model referred to as Susceptible-Exposed-Infectious-Recovered (SEIR) model. Equally, addition or alteration of attributes transition is possible[113]. For example, individuals may lose their acquired post-recovery immunity over time, resulting in changing their status from the removed to the susceptible, thereby yielding the Susceptible-Infectious-Recovered-Susceptible (SIRS) model[114]. Similarly, the removed state can entirely be left out of the model if the infectious agent under study does not trigger the production of any form of post-recovery immunity, yielding Susceptible-Infectious-Susceptible (SIS) model[115].

One disadvantage of the basic SIR model is that it cannot discriminate whether an infected individual develops symptoms, even though this could be an essential transmission factor[109]. For instance, individuals infected with airborne respiratory pathogens are more likely to spread the infection if they develop frequent coughing and sneezing symptoms. Similarly, it is necessary to account for asymptomatic individuals for diseases in which asymptomatic infection (carriage) is the fundamental transmission driver, such as meningococcal or pneumococcal disease. Irrespective of the model specification, individuals are primarily assigned to a group based on specific health attributes, which could change from time to time. In the last ten years, modelers have faced increasing hurdles, the most important of which is the growing availability of genomic and other 'omics' data generated for diagnostic and surveillance, which has reformed the field of risk assessment[116].

However, recent advances in computer algorithms and machine learning technology offer researchers an efficient alternative that overcomes these challenges[117].

WGS, MACHINE LEARNING AND MICROBIAL RISK ASSESSMENT

Establishing the links between WGS or metagenomics data sets and specific risk indicators is especially important. However, the complex nature of genomic data concerning the number of microbial isolates remains a significant challenge, especially in applying conventional statistical tools[82]. Most microbial risk assessment models cannot discriminate strains in terms of their differences in resistance and virulence. Interestingly, machine learning technology and other novel models currently deployed in microbial risk assessment can analyze large data sets while accurately predicting the risk/in a population[118]. Machine learning algorithms are developed and employed for risk assessment. Over time, these algorithms are improved for better performance. These technologies can identify a combination of factors that allows the prediction of risk outcomes, thereby making risk assessment from big data sets more sensitive and reliable. Additionally, conventional risk assessment models usually use intermediate genetic interactions[119].

On the other hand, machine learning algorithms consider personal effects, which rely on interactions between environmental and genetic factors. Machine learning algorithms allow simultaneous prediction and interpretation using big data sets. Consequently, it is possible to unveil a particular phenotype and predict the presence of the protein from a sequence. With machine learning methods, it is also possible to carry out a microbial risk assessment with the flexibility to certain genetic acquired variations, which could favor the timely identification of strains with novel resistance or virulence determinants[118]. The applications of machine learning technology in genomics and as a placement for the classical genome-wide association studies have proliferated in recent years. Far-reaching disease indicators have been studied through their application to gene expression data, where computer algorithms learn to discriminate between various disease phenotypes. Other successful applications of machine learning algorithms in health and disease include a better understanding of the relationship between patient genotypes, gene-expression-related phenotypes, and patient outcomes in cancer research, as well as the discovery of regions in bacterial genomes code for antibiotic resistance. The application of machine learning algorithms in risk assessment using WGS data has been described. WGS data becomes a powerful tool for microbial source tracking and risk assessment when analyzed using an appropriate source tracking algorithm.

CONCLUSION

In conclusion, the evidence reviewed here provides valuable information on the various medically necessary *Campylobacter* spp, their mechanism of resistance, important reservoirs, and most importantly, how advanced molecular techniques are deployed in microbial risk assessment and source tracking. In particular, the limitations of conventional methods, which include time-consumption, poor sensitivity and specificity on the one hand, and the superiority of WGS and machine learning technology, which include high reliability and robustness, on the other hand, have been explored. The application of machine learning and NGS technologies offer massive potential since they can be deployed in combination to track sources of outbreaks and predict risks. If timely deployed, they could help tackle outbreaks from their sources, thereby minimizing casualties and other impacts. Noteworthy, these technologies, despite their numerous advantages, their deployment in resource-limited settings is constrained by factors such as lack of expertise and the cost.

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AUTHORS' CONTRIBUTION

Bashar Haruna Gulumbe: conceptualization, drafting of the manuscript, revision of the manuscript, and approval of the final draft. **Abbas Yusuf Bazata**: conceptualization, drafting of the manuscript, revision of the manuscript, and approval of the final draft. **Musbahu Abdullahi Bagwai**: drafting of the manuscript, revision of the manuscript, and approval of the final draft.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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