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Fourier transform infrared spectroscopy; can it be used as a rapid typing method of *Neisseria gonorrhoeae*?

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ABSTRACT

Background: Typing of *Neisseria gonorrhoeae* is necessary for epidemiologic surveillance, while time consuming and resource intensive. Fourier transform infrared (FTIR) spectroscopy has shown promising results when typing several bacterial species. This study investigates whether FTIR spectroscopy can be used as a rapid method for typing clinical *N. gonorrhoeae* isolates, comparing FTIR spectroscopy to multi locus sequence typing (MLST), *N. gonorrhoeae* multi antigen sequence typing (NG-MAST) and whole genome sequencing (WGS).

Methods: Sixty consecutive isolates from a venereology clinic and three isolates from an outbreak were included. Isolates were analysed with FTIR spectroscopy on the IR Biotyper system (Bruker Daltonik) with the IR Biotyper software (version 2.1) with default analysis settings (spectral range 1300–800 cm^{-1}). Four technical replicates of each isolate were analysed in three different runs. The output was a hierarchical cluster analysis (HCA) presented as a dendrogram; a tree-like overview of how closely different isolates are related. FTIR spectroscopy was compared to MLST, NG-MAST and WGS to see if the FTIR spectroscopy-dendrogram grouped the isolates in the same clusters.

Results: Fifty-one out of 60 isolates, and the three outbreak isolates, produced at least one spectrum in each run and were included. No agreement between FTIR spectroscopy and MLST or NG-MAST or WGS was shown. The FTIR spectroscopy-dendrogram failed to cluster the outbreak isolates.

Conclusion: FTIR spectroscopy (spectral range 1300–800 cm^{-1}) is not yet suitable for epidemiologic typing of *N. gonorrhoeae*. Absence of a capsule as well as phase- and antigenic variation of carbohydrate surface structures of the gonococcal cell wall may contribute to our findings. Future studies should include analysis of a wider range of the spectrum recorded (4000-500 cm^{-1}), and should also explore further mathematical analytic approaches of the similarity between spectra.

1. Introduction

It is a public health priority to improve the surveillance of the epidemiological characteristics and the transmission of gonorrhoea (World Health Organization, 2012). Targeted control interventions will in turn decrease the spread of antibiotic resistant gonococci, which is on the World Health Organization's (WHO) priority list for development of new antibiotics. One of the main goals in WHO's action plan is to control the spread of *N. gonorrhoeae*(World Health Organization, 2012). A simple and rapid typing method, to identify outbreaks more quickly is warranted.

To develop new strategies for prevention and control of gonorrhoea, we have to understand who contracts the infection, where (in which city or country) the patients get infected and what characterizes the gonococci which cause the infections. Specific patient groups can be infected by type-specific gonococci. For instance, men who have sex with men

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may be infected with gonococci that differ from gonococci circulating in a group of men who have sex with women. Specific types of gonococci can also dominate in different geographical areas.

As of today, the surveillance of gonorrhoea uses different typing methods. The most established typing methods for *N. gonorrhoeae* are multi locus sequence typing (MLST)(Maiden, 2006) and *N. gonorrhoeae* multi antigen sequence typing (NG-MAST)(Unemo and Dillon, 2011). MLST uses the sequences of seven housekeeping genes to characterize isolates of *N. gonorrhoeae*. NG-MAST is based on the sequences of two hypervariable genes; *porB* (a porin) and *tbpB* (a transferrin binding protein), both encode superficial antigens of the gonococcus. NG-MAST has shown to be more versatile for local outbreak investigations, while MLST seems to be a good choice to describe population structure in global epidemiological studies (Ilina et al., 2010). Typing based on whole genome sequencing is a potential gold standard with a better resolution compared to both MLST and NG-MAST(De Silva et al., 2016; Didelot et al., 2016; Harris et al., 2018; Kwong et al., 2018; Town et al., 2018). WGS can provide in silico MLST and NG-MAST. Today MLST and NG-MAST based on WGS in many places have replaced conventional MLST and NG-MAST. Nevertheless, common to all the established typing methods, is that they are both resource intensive and time consuming; it takes several days before the results are ready.

Fourier transform infrared (FTIR) spectroscopy on the IR Biotyper system (spectral range 1300–800 cm^{-1}) has shown high agreement with clustering based on whole genome sequencing (WGS) of *Klebsiella pneumoniae*(Dinkelacker et al., 2018; Rodrigues et al., 2020). FTIR spectroscopy has shown promising results in order to predict the capsular serotype of *Streptococcus pneumoniae*(Burckhardt et al., 2019) and to discriminate serovars of *Listeria monocytogenes* (Rebuffo-Scheer et al., 2007). FTIR spectroscopy on the IR Biotyper system (spectral range 1300–800 cm^{-1}) has also shown to cluster *Enterobacter cloacae*, *K. pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* in hospital outbreaks(Martak et al., 2019).

FTIR spectroscopy uses infrared light that causes vibrations of the different chemical compounds of the bacterial cell. The bacterial cell wall consists of different functional groups, such as proteins, lipids and carbohydrates. Each of these groups will provide different characteristic spectral peaks based on the transmission of infrared radiation (Novais et al., 2018).

The transmitted infrared radiation of a bacterial sample provides a spectrum, which is like a specific fingerprint(Novais et al., 2018). Five different spectral windows correspond to the absorption expressed in wavenumbers (cm^{-1}); 1) lipids (3000–2800 cm^{-1}), 2) proteins and peptides (1800–1500 cm^{-1}), 3) a mixed region with information of proteins, fatty acids and phosphate-carrying compounds (1500–1200 cm^{-1}), 4) polysaccharides (1200–900 cm^{-1}) and 5) a fingerprint region showing some specific spectral patterns not yet assigned to functional groups or cellular components (900–700 cm^{-1})(Novais et al., 2018). To type bacteria, the spectral range of 1200 to 900 cm^{-1} is the most used, which includes the characteristic spectral peaks of carbohydrates present within the bacterial cell wall(Novais et al., 2018).

Since FTIR spectroscopy has shown a high discriminatory power for several bacterial species, and the results can be ready after hours instead of days, we wanted to explore it as a viable alternative to molecular methods for the typing of *Neisseria gonorrhoeae*. In this study, we investigated whether FTIR spectroscopy can be used as a rapid typing method of 63 isolates of *N. gonorrhoeae* in comparison to MLST, NG-MAST and WGS.

2. Materials and methods

2.1. N. gonorrhoeae isolates

1) **Routine isolates:** Sixty consecutive isolates of *N. gonorrhoeae* from patients attending the venereology clinic of Oslo University Hospital, during a 3 months period from August 2016 – November 2016 were included. Strains were recovered from swab samples from urethra, cervix/vagina, anus and pharynx.
2) **Outbreak isolates:** Three isolates of *N. gonorrhoeae* from a defined gonorrhoea outbreak from a nearby municipality were included.

The *N. gonorrhoeae* isolates were stored at −80 °C. Prior to FTIR spectroscopy typing, strains were grown on chocolate agar plates and incubated at 37 °C in 5% CO_2. The included isolates were re-identified at the species level (score ≥ 2.0 MBT-ROU v.7.0.0.0 (DB7311)) using matrix-assisted laser desorption ionization time-of flight (MALDI-TOF) mass spectrometry (Bruker Daltonik GmbH).

Subcultures were incubated in 37 °C in 5% CO_2 for 24 (±0.5) hours (as recommended by the manufacturer's instructions, IR Biotyper user manual), before FTIR spectroscopy (FTIR transmission, IR spectrometer, Bruker Daltonik GmbH).

A loopful (1 μl loop) of bacteria was added to 50 μl of 70% (vol/vol) ethanol in Eppendorf vials. Metal rods were added for homogenization (Bruker Daltonik GmbH). After 10 mins vortexing, 50 μl of deionized H_2O was added. 15 μl of the resulting suspension of bacteria was placed on a silicon sample plate (Bruker Daltonik GmbH) and dried at 37 °C for 10–15 min. Each isolate was analysed in four technical replicates in several independent runs on different days on the IR Biotyper system with the IR Biotyper software (version 2.1, Bruker Daltonik GmbH) with default analysis settings (as recommended by the manufacturer's instructions, IR Biotyper user manual). If one isolate produced no spectra of any replicates in one run, the rest of the suspension was set up in duplicate. Turn-around time for the FTIR spectroscopy acquisition for one run with 60 isolates was 6–8 h.

Twelve μl of the test standards (Infrared Test Standards, IRTS 1 and 2, Bruker Daltonik GmbH) were placed in duplicates on each sample plate. A successful measurement of the test standards was mandatory for further spectra acquisition (as recommended by the manufacturer's instructions, IR Biotyper user manual).

Three independent analyses were performed:

1) **"Standard analysis":** Three isolates (isolate number 11, 52 and 59) from the routine isolates were set up in four technical replicates in five runs. The five runs were set up separately on five different days. Standard analysis was performed to investigate variation between runs (biological variance).
2) **"Routine-analysis":** Sixty isolates from the venereology clinic were analysed with four technical replicates in three runs. The three runs were set up separately on three different days.
3) **"Outbreak-analysis":** Three isolates from the outbreak were analysed together with six isolates from the routine analysis from the venereology clinic, in four technical replicates, in three runs. The three runs were set up separately on three different days. The six isolates from the venereology clinic differed in MLST, NG-MAST and WGS.

2.2. FTIR spectroscopy

IR Biotyper uses transmission FTIR spectroscopy. The spectral range recorded was 4000 to 500 cm^{-1} and the spectral resolution was 6 cm^{-1} (default settings). A background measurement was performed before each sample spot (replicate). We did 32 scans for the background and 32 scans for the sample (default settings). It took round about 40 s per spot to measure background and sample.

2.3. Analysis of spectra

The software (IR Biotyper version 2.1) processes the spectra based on the second derivative of the 1300–800 cm^{-1} wavenumber range of the spectra, which is the manufacturer's default setting (other publications report the range 1200–900 cm^{-1}(Novais et al., 2018) (Quintelas et al., 2018)). The spectra were vector-normalized after calculating the

derivative and cutting to 1300–800 cm^{-1}.

The spectra of an isolate acquired in one run, are named the "technical replicates". The technical replicates of an isolate (acquired in one run) are summarized to a "biological replicate". Then, the spectra of the isolate's biological replicates (acquired in different runs) are summarized to a total "isolate spectrum".

A spectral distance value arises from a comparison between the results of two spectra. Each spectrum is a multidimensional vector with 500 + dimensions. Different ways to determine similarity between spectra exist, we used the "Euclidean distance" (ED) to calculate the distance between the normalized vectors, the equivalent to measuring the distance between two objects in the real world. ED was calculated as follows: Similarity (%) = (1-ED) x 100. A matrix was generated from a calculation of the similarity between all isolate spectra. The matrix/similarity values were used to cluster the isolates with the average linkage algorithm by the IR Biotyper software (version 2.1) and sort them in a hierarchical cluster analysis (HCA). The output of the HCA is a dendrogram with a tree-like overview of how close different isolates are related. A spectral distance of 0 means that the two spectra are identical.

2.4. WGS, MLST and NG-MAST

DNA was extracted using MagNA Pure 96 (Roche), KAPA HyperPlus (Roche) was used for NGS DNA library prep, and DNA libraries were sequenced using MiSeq 2 × 300 v3 kit (illumina) following the manufacturers suggestions.

Paired end sequence reads were trimmed and adapters removed using trimmomatic v0.36 (at default settings(Bolger et al., 2014)), trimmed sequence reads were assembled using SPAdes v3.12.0 (with following adjustments to the default settings; careful mode on and read coverage cut-off was set to automatic computation of coverage threshold)(Nurk et al., 2013). Resulting contigs were filtered using an in-house python-script removing contigs smaller than 500 bp and with contig coverage below 2.0. Core genome multi-alignment and SNP tree were generated from the filtered contigs using Parsnp v1.2 (from the Harvest suite – with the following adjustments to the default settings; curated genome directory to include all genomes, and enabled filtering of SNPs located in PhiPack identified regions of recombination (Treangen et al., 2014). Regions of recombinations were removed using Gubbins v1.4.5 (default settings (Croucher et al., 2015)).

MLST(Jolley and Maiden, 2010) was determined from the filtered contigs described above using mlst v2.15 (https://github.com/tseemann/mlst) using database updated 2018-09-04, NG-MAST(Martin et al., 2004) was determined using ngmaster v0.5.5 (https://github.com/MDU-PHL/ngmaster) (ref Microbiological Diagnostic Unit – Public Health Laboratory (MDU-PHL) – Melbourne, Australia) using database updated 2018-11-14.

Visualization of trees and compilation of data using R v3.5.1 with the package *ggtree* v1.12.7(Yu et al., 2018), comparison of HCA dendrogram and the core genome SNP tree was done using *phytools* v0.7–90(Revell, 2012).

3. Results

Three independent analyses were performed. First, a standard-analysis with three isolates was set up to investigate variations between runs (biological variance). Second, in the routine-analysis, 60 isolates were investigated to see if FTIR spectroscopy grouped the isolates in the same manner as MLST, NG-MAST and WGS. Finally, three isolates from a known outbreak were analysed together with 6 isolates from the routine-analysis to see if FTIR spectroscopy could group the isolates from the outbreak.

3.1. The standard analysis

Three standard isolates were analysed with four technical replicates in five different runs. Using the optimized cut-off from the manufacturer, the spectra from the three different isolates grouped in three separate clusters in three out of five runs. Two runs did not manage to cluster the three standard isolates in three separate groups (Fig. 1).

3.2. The routine-analysis

Sixty isolates from the venereology clinic were analysed with four technical replicates on three different runs.

The median number of spectra produced per isolate per run was three (range 0–4). Twelve isolates had four replicates that did not produce any spectra in one run. We used the rest of the suspension and repeated these isolates in duplicates in an extra run. Three of the repeated isolates produced spectra in the extra run, three isolates did not produce any spectra, and six isolates were technically unsuccessful in the repeated run. Summarized; $n = 9$ isolates had no replicates that produced any spectra in one run. These were excluded (Table 1).

A dendrogram visualizes the results of the 51 included *N. gonorrhoeae* isolates and shows the relations of the average spectra in a tree-like overview. Isolates with the same MLST or NG-MAST did not cluster together in the dendrogram (Fig. 2). Clustering by FTIR spectroscopy and WGS showed no agreement (Fig. 3).

3.3. The outbreak-analysis

The outbreak isolates were analysed together with six isolates from the "routine analysis". Four technical replicates on three different runs on separate days were analysed.

The three isolates from the outbreak (61, 62 and 63) belonged to MLST 7363 and NG-MAST 13878. One of the six isolates from the venereology clinic had the same MLST (7363), but a different NG MAST (8503). The remaining five isolates from the "routine-analysis" differed in MLST and NG-MAST.

FTIR spectroscopy did not manage to cluster the outbreak isolates in one cluster (Fig. 4), in contrast to WGS (core genome SNP tree) (Fig. 5).

3.4. Cut-off

The manufacturer presents alternative methods to calculate a cut-off value for clustering in the dendrogram. One recommended method is to calculate the optimized product of Simpson's Diversity index (SDI) and mean coherence (mc). SDI measures the discriminatory power. An SDI of zero means that all spectra group in one cluster, an SDI of value 1 means that every single spectrum forms its own cluster. The software calculates a coherence value (mc) for each isolate ID. A mc value of zero means that every single spectrum of an isolate ID is in a different cluster. High coherence is achieved when spectra that belong together are kept in one cluster. A mc value of one means that all single spectrums of an isolate ID are in the same cluster. When maximizing the product of SDI and mc, one will achieve both good discrimination and coherence.

The manufacturer has also by personal message presented alternative methods to calculate a cut-off value; distance matrix histogram analysis and isolate variance using quantiles.

The distance matrix histogram analysis is based on within isolate distances and between isolate distances. If within and between isolate distances are presented in a histogram, ideally a gap between small (within isolate distances) and large (between isolate distances) should appear and give a hint for the right cut off value. This method does not work with all species (Fig. S1, supplementary).

The other method, isolate variance using quantiles, is also based on the distance matrix, but only the distances between spectra of the same isolate are taken into account (within isolate distances). By calculating quantiles to estimate confidence intervals, and use the cut off range values from 90 to 99%, this should give a hint for a cut off value (Fig. S2, supplementary).

A simpler approach to define a suitable cut-off is to reason that FTIR

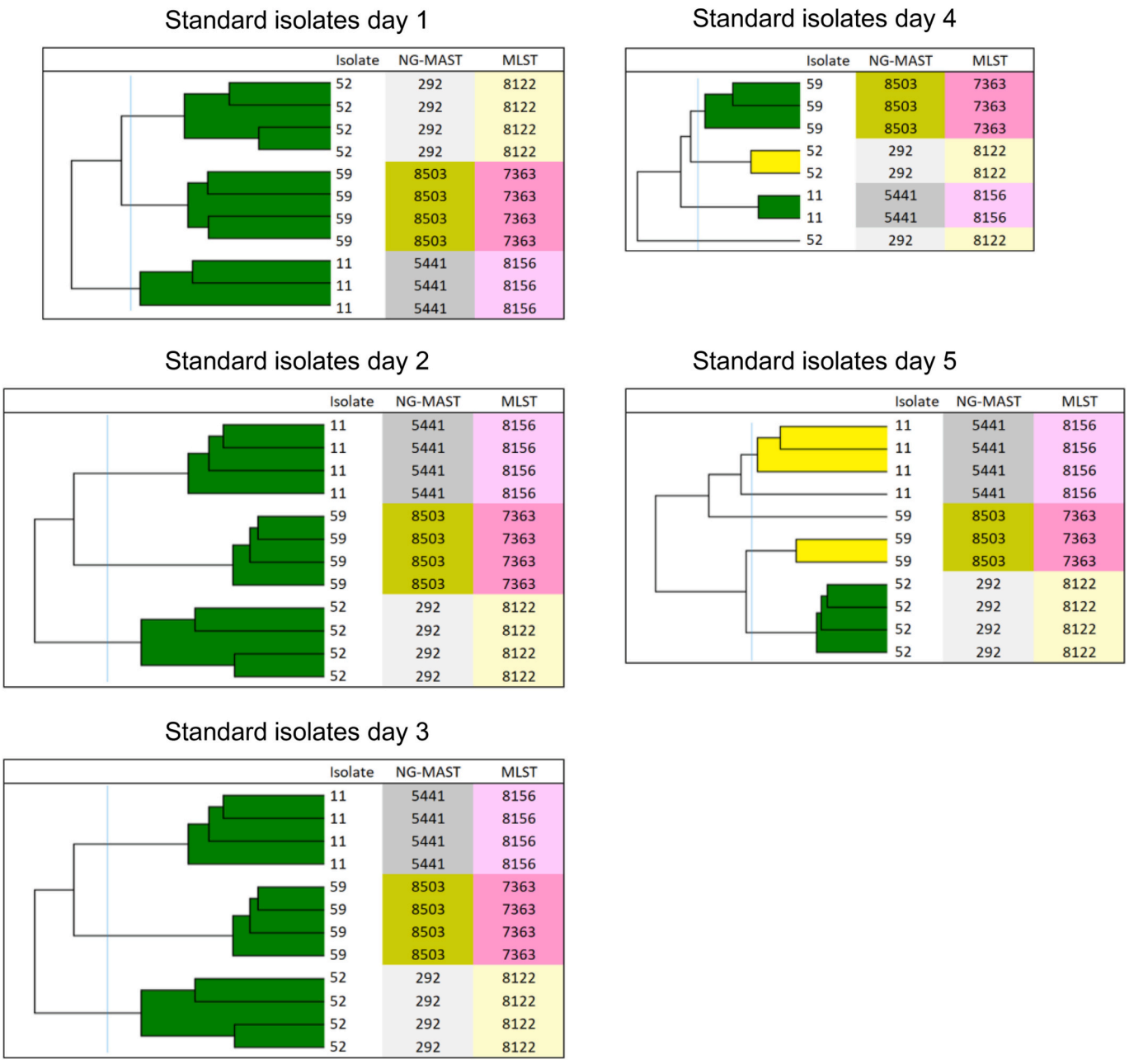

Fig. 1. Dendrogram of spectra of the three standard isolates analysed on five separate days, with their corresponding NG-MAST and MLST. The dendrogram is a visualization of the hierarchical cluster analysis, a tree-like overview, based on mathematical calculations of spectral similarity values between isolates. Each replicate spectra of the isolates (not the summary/average spectra) is visualized in the dendrogram. The yellow colour in the tree indicates that the technical replicates of the same isolate did not manage to group in one cluster. Green colour indicates successful clustering of the technical replicates belonging to the same isolate. Light blue vertical line represents the optimized cut-off from the manufacturer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Isolates and spectra per run for the routine-analysis.

	Included isolates	Excluded isolates	Total number of spectra produced	Median number of spectra produced per isolate
Run1	$n = 60$	$n = 1$	152	2
Run2	$n = 59$	$n = 4$	162	3
Run3	$n = 55$	n = 4	141	2
Total	$n = 51$	n = 9	455	3

spectra of genetically identical isolates, for example isolates from a known outbreak, will group in one cluster. The spectral distance value, which manage to cluster the known genetically similar isolates in one group, will then represent a reasonable cut-off value. Our analyses did not manage to group the three outbreak isolates, which we knew shared the same MLST and NG-MAST, and grouped in one cluster by WGS. Based on this, we decided that a calculation of a cut-off made no sense.

4. Discussion

The main objective was to determine if the rapid typing method FTIR spectroscopy on the IR Biotyper system (spectral range 1300–800 cm^{-1})

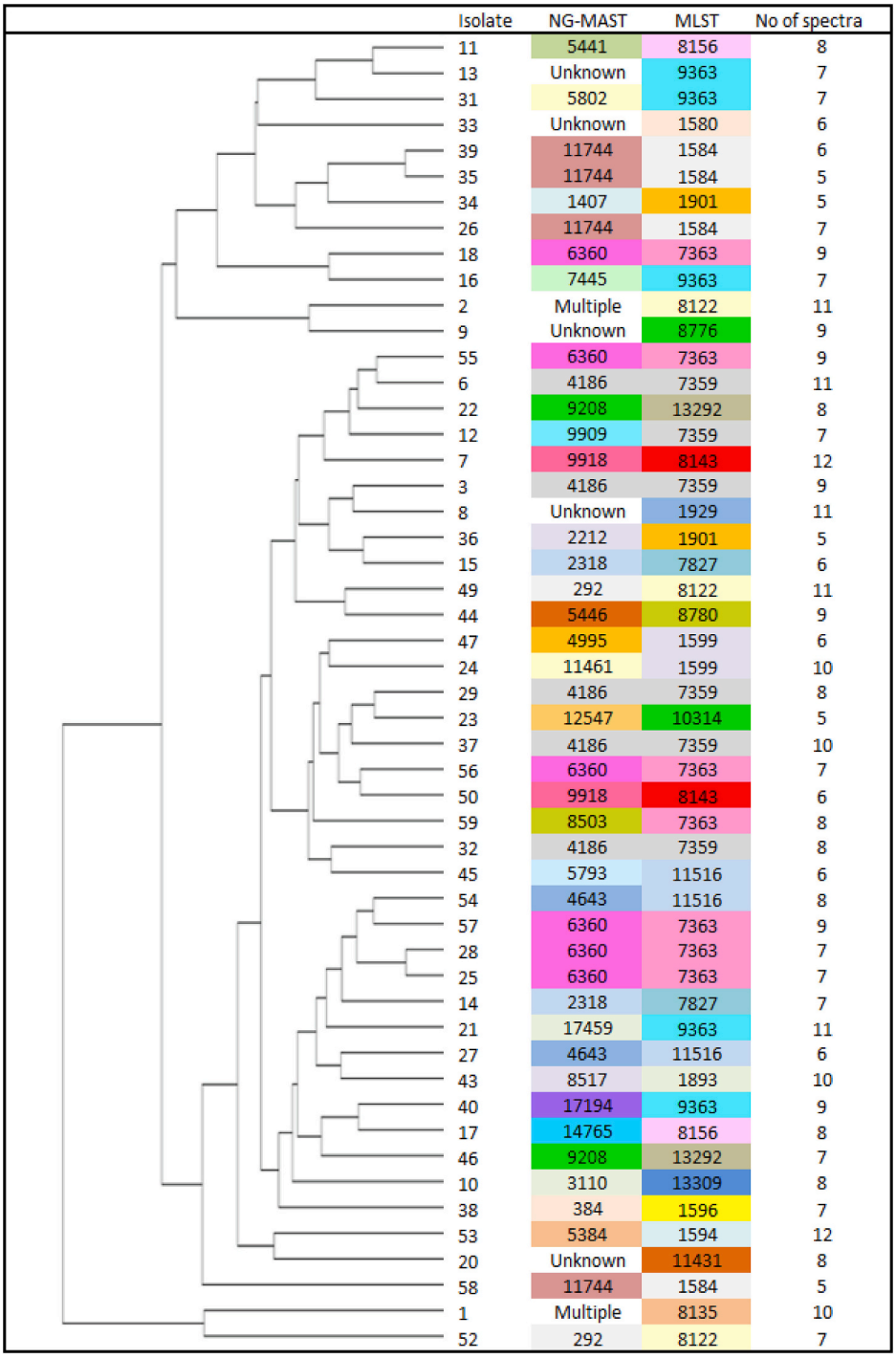

Fig. 2. Calculation of the similarity between all isolate spectra generated a matrix. The matrix/similarity values were used to cluster the isolates with the average linkage algorithm by the IR Biotyper software (version 2.1). Dendrogram (HCA output) of the average spectra of the 51 included isolates, NG-MAST, MLST and total number of spectra per isolate.

is suitable for the typing of *N. gonorrhoeae*, and compare it to the more time consuming but established typing methods MLST, NG-MAST and WGS. Our second objective was to investigate whether FTIR spectroscopy can be used as a tool to detect outbreaks of gonorrhoea. FTIR spectroscopy on the IR Biotyper system (spectral range 1300–800 cm^{-1}) could discriminate between *N. gonorrhoeae* isolates, but the correlation to MLST, NG-MAST and WGS was poor (Figs. 2 and 3). Importantly, the three isolates from a defined outbreak did not group in one cluster in the FTIR spectroscopy dendrogram (Fig. 4).

In contrast to the established typing methods MLST, NG-MAST and WGS, FTIR spectroscopy is rapid (6–8 h for one run with 60 isolates), easy-to-apply and inexpensive. However, some technical challenges of FTIR spectroscopy do exist:

1) The need for all strains to be analysed simultaneously: It is crucial that strains are incubated, prepared and acquired under the same experimental conditions (e.g. room temperature and humidity). As far as we know this is the first time FTIR spectroscopy is investigated on *N. gonorrhoeae*, and we did not have a preformed database of FTIR spectra of *N. gonorrhoeae*.

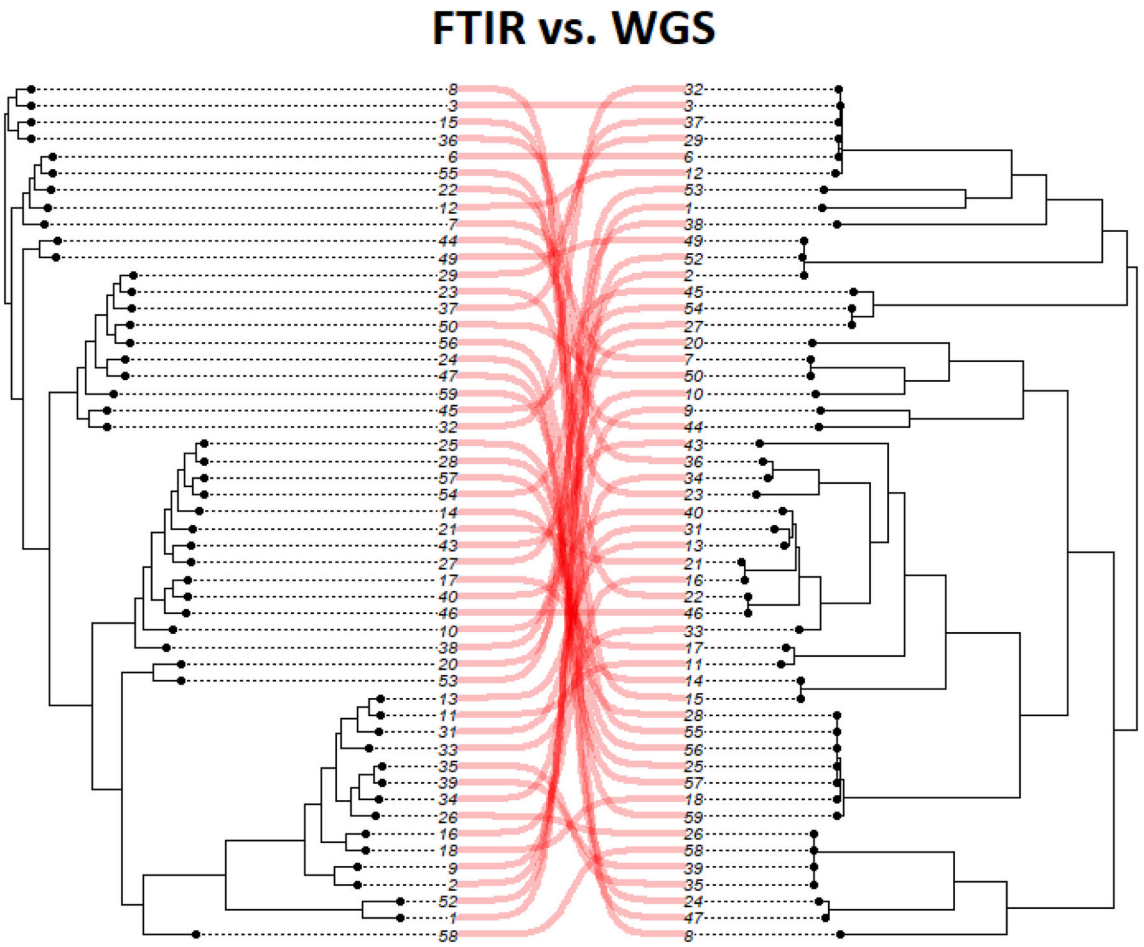

Fig. 3. Clustering by FTIR spectroscopy vs WGS. Red lines are drawn between identical isolate numbers. No systematic agreement of the clusters between the FTIR spectroscopy dendrogram and the core genome SNP tree was shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

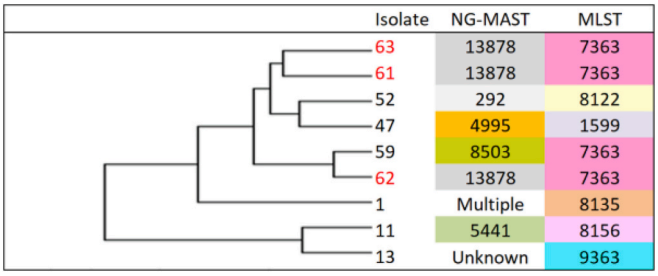

Fig. 4. Dendrogram including average spectra from three outbreak-isolates (marked by red numbers), together with six other isolates from the "routine analysis" which differed in MLST and NGMAST. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2) The need for replica testing to check for technical variance: Four replicates of each isolate were set up in each run. There were enough suspension to set up six replicates of each isolate per run. We could have done this to improve our results, but then more spots on the plates would be used, and more plates would have to be analysed.
3) Testing in several consecutive runs controlling biological variance/reproducibility. Incubation and preparation has a bigger impact on the results compared to technical factors such as drying of the spots on the plate.
4) For some bacterial species, a reasonable method to calculate a cut-off value may be challenging.

Because of the technical challenges and the concerns related to culture media, incubation time, temperature and hygrometry leading to variation between different runs on different days, a standard analysis was performed. Three isolates, which differed in NG-MAST and MLST were set up in five runs on separate days. The spectra from the three different standard isolates grouped in three separate clusters in three out of five runs, and indicated a good reproducibility of the FTIR spectroscopy analysis. The unsuccessful clustering in two runs are assumed to be caused by technical factors as too thick or too thin spots or bubbles introducing noise and increasing the technical variance between replicates.

FTIR spectroscopy on the IR Biotyper system (spectral range 1300–800 cm^{-1}) has shown high discriminatory power and good correlation to clustering based on WGS in a study of 68 isolates of *K. pneumoniae*(Dinkelacker et al., 2018). Further, promising results to detect hospital outbreaks with *E. cloacae*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*(Martak et al., 2019) has been reported. FTIR spectroscopy has also shown promising results in order to predict the capsular serotype of *S. pneumoniae*(Burckhardt et al., 2019). It is noteworthy that, a capsule is common to all of these bacteria. Biochemical and genetic differences in the structure of capsular polysaccharides are well known and used in serotyping of capsulated bacteria. *N. gonorrhoeae* is missing a capsule, and this may be an important contributing factor to our findings showing no correlation of FTIR

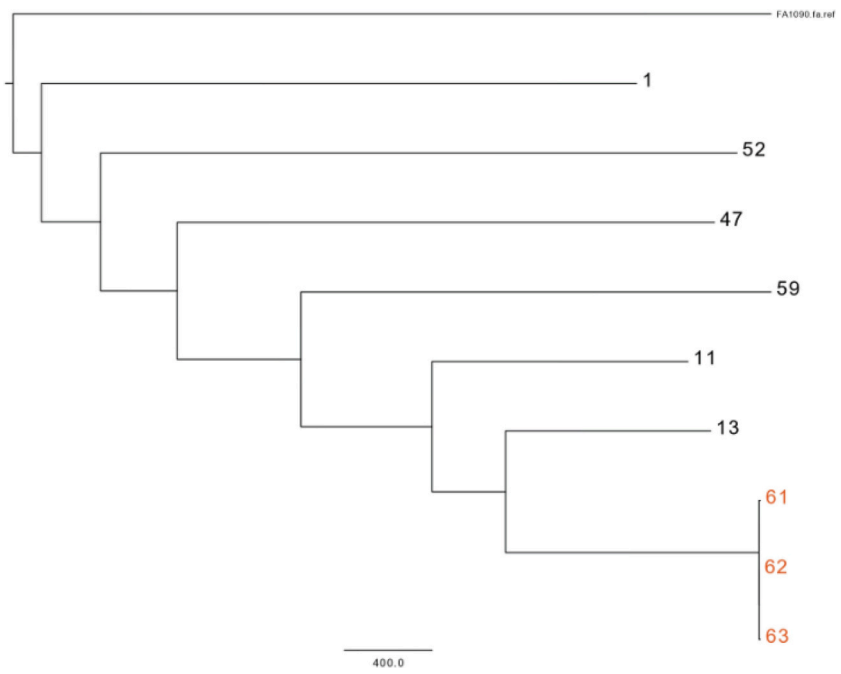

Fig. 5. Clustering made by WGS (core genome SNP). The three outbreak isolates grouped in one cluster.

spectroscopy (spectral range 1300–800 cm^{-1}) with MLST, NG-MAST and WGS. However, FTIR spectroscopy has shown promising results to discriminate serovars of *L. monocytogenes* which has no capsule(Rebuffo-Scheer et al., 2007).

FTIR spectroscopy on the IR Biotyper system (spectral range 1300–800 cm^{-1}) is a phenotypic typing method as it analyses and produces spectra of polysaccharides present in the cell wall of the bacteria, in contrast to genotypic methods, such as NG-MAST, MLST and WGS. Surface molecules of the cell wall of the gonococcus are often antigenic variable and epitopes are modified through antigenic or phase variation (Gulati et al., 2019). Phase variation is caused by switching of expression of a given phenotype; it can be switched ON or OFF. Antigenic variation results in multiple forms of an antigen by changes in genes that alter the peptide sequence or glycan structure rather than the expression level (Rotman and Seifert, 2014).

As far as we know, this is the first report of FTIR spectroscopy of *N. gonorrhoeae,* and the exact polysaccharides and other constituents of the gonococcus producing the spectra in the wavelength 800–1300 cm^{-1} is unknown. We suggest the spectra represents the carbohydrate constituents of the cell wall of the gonococcus. Structures composed of carbohydrates in the gonococcal cell wall include virulence factors, peptidoglycan and glycoproteins. *N. gonorrhoeae* has several known virulence factors in its cell wall(Quillin and Seifert, 2018); type IV pili, lipooligosaccharide (LOS), porin and opacity (Opa) proteins. Of these, LOS and Type IV pili contains carbohydrate constituents. Importantly, LOS has been proposed as a potential vaccine candidate, because they are densely represented on the bacterial surface(Gulati et al., 2019). It is suggested that 75% of the outer membrane of Neisseria is made up of LOS(Mubaiwa et al., 2017). Lipooligosaccharide contains three oligosaccharide chains(Mubaiwa et al., 2017). The individual sugars that constitutes the oligosaccharide chains, are added by glycosyltransferases. Notably, the glycosyltransferases are phase-variable (Mubaiwa et al., 2017) and this may result in variable lengths of the oligosaccharide chain within and between strains of gonococci(Apicella et al., 1987; Mubaiwa et al., 2017). When gonococci are grown in culture, the phase variation of LOS occurs at a frequency of 10^{-2} - 10^{-3} (Gulati et al., 2019). We hypothesize that this phenotypic switch, which creates an ever-changing bacterial surface, hampers the use of LOS as a marker in typing with FTIR spectroscopy, and also makes LOS a less desirable vaccine candidate(Gulati et al., 2019).

Further, *N. gonorrhoeae* has glycosylated proteins on its outer membrane and in the periplasm(Vik et al., 2009). We suggest that FTIR spectroscopy (spectral range 1300–800 cm^{-1}) also detects these glycosylated proteins, as infrared spectroscopy has been used to detect glycosyl groups on proteins(Khajehpour et al., 2006). Of the eleven glycoproteins of *N. gonorrhoeae*(Vik et al., 2009), the PilE subunit of pilin and the nitrite reductase AniA are the best characterized(Mubaiwa et al., 2017). PilE is the most abundant glycoprotein of *N. gonorrhoeae* (Vik et al., 2009), and thousands of copies of PilE make up the virulence factor Type IV pili(Craig and Altindal, 2019). Previous studies have suggested that glycans on PilE might be antigenically variable both within and between strains(Børud et al., 2010). Glycosylation of pilin involves multiple glycosylation genes (*pgl*), and three of these glycosyltransferase genes (*pglA, pglE* and *pglH*) are known to be phase-variable (Børud et al., 2011; Mubaiwa et al., 2017). Studies have indicated that one *N. gonorrhoeae* strain can express at least six distinct glycoforms (Børud et al., 2011; Børud et al., 2010).

Another polysaccharide, poly-*N*-acetylglucosamine (PNAG) has been identified as a conserved surface polysaccharide produced by different bacteria, including *N. gonorrhoeae.* PNAG is a possible vaccine candidate target, and since this is a polysaccharide, we suggest that PNAG also may be detected and discriminated by FTIR spectroscopy (spectral range 1300–800 cm^{-1}) (Cywes-Bentley et al., 2013).

FTIR spectroscopy may be a promising tool to discriminate closely related strains of gonococci, although we show a poor correlation to the established typing methods. We used only the spectral range of 1300 to 800 cm^{-1}, which includes spectral peaks of carbohydrates present in the cell wall of the gonococcus. Spectral regions associated with other chemical compounds such as lipids, proteins, peptides and amides were not investigated by the version 2.1 of the software. Further investigations should include a wider range of the spectal wavelengths. Likewise, future research should also include different mathematical analytic approaches of the similarity between spectra (principal component analysis and linear discriminant analysis).

Despite we do not exactly know what the FTIR spectroscopy detects and discriminates in the cell wall of the gonococcus. FTIR spectroscopy may contribute in the detection and characterization of gonococcal antigens and to the development of a gonococcal vaccine.

5. Conclusions

The discrimination of clinical *Neisseria gonorrhoeae* isolates by FTIR spectroscopy on the IR Biotyper system (spectral range 1300–800 cm^{-1}) did not show agreement with NG-MAST, MLST and WGS, and FTIR spectroscopy was not able to cluster three isolates from a known outbreak in one group. Absence of a capsule as well as phase- and antigenic variation of carbohydrate surface structures of the gonococcal cell wall may contribute to our findings. However, FTIR spectroscopy could discriminate between different isolates of *N. gonorrhoeae* with high resolution. Future research should include analysis of a wider range of the spectrum recorded (4000 to 500 cm^{-1}), and also include different analytic approaches of the similarity between spectra. Presently, we cannot recommend FTIR spectroscopy on the IR Biotyper system (spectral range 1300–800 cm^{-1}) as a rapid epidemiologic typing method for *N. gonorrhoeae*. FTIR spectroscopy on other Neisseria species as *Neisseria meningitidis* having a capsule defining different serotypes, should also be investigated.

Glossary

FTIR spectroscopy Fourier transform infrared spectroscopy
N. gonorrhoeae *Neisseria gonorrhoeae*
MLST Multi locus sequence typing
NG-MAST *Neisseria gonorrhoeae* multi antigen sequence typing
WGS Whole genome sequencing
HCA Hierarchical cluster analysis
WHO World Health Organization
K. pneumoniae *Klebsiella pneumoniae*
CO_2 Carbon dioxide
MALDI-TOF Matrix-assisted laser desorption ionization time-of flight
µl Microliter
H_2O Dihydrogen monoxide, water
IRTS Infrared test standards
Cm centimetre
ED Euclidean distance
DNA Deoxyribonucleic acid
Bp Base pair
SNP Single nucleotide polymorphism
SDI Simpson‘s Diversity Index
Mc Mean coherence
ID Identification
E. cloacae *Enterobacter cloacae*
P. aeruginosa *Pseudomonas aeruginosa*
A. baumannii *Acinetobacter baumannii*
S. pneumoniae *Streptococcus pneumoniae*
L. monocytogenes *Listeria monocytogenes*
LOS Lipooligosaccharide
Opa Opacity
PNAG poly-*N*-acetylglucosamine
N. meningitidis *Neisseria meningitidis*

Ethics approval and consent to participate

A written approval for the study was obtained from Oslo University Hospital‘s Privacy and Data Protection Officer (18/18595). The need for consent was waived by the Oslo University Hospital‘s Privacy and Data Protection Officer (18/18595).

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CRediT authorship contribution statement

Linn Merete Brendefur Corwin: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **André Ingebretsen:** Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization. **Patricia Campbell:** Resources, Writing – review & editing. **Kristian Alfsnes:** Formal analysis, Resources, Data curation, Writing – review & editing, Visualization. **Fredrik Müller:** Writing – review & editing. **Norman Mauder:** Methodology, Software, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Funding acquisition. **Michael Koomey:** Writing – review & editing. **Jørgen Vildershøj Bjørnholt:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

Norman Mauder is employed at Bruker Daltonik GmbH. The other authors have no relevant financial or non-financial interests to disclose.

Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request. Sequence data downloaded in the European Nucleotide Archive (ENA).

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Appendix A. Supplementary data

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