ABSTRACT

Background: Neisseria gonorrhoeae (N. gonorrhoeae) causes urogenital infection called as gonorrhea. The infection is often asymptomatic in women. High-risk populations such as sex workers and antimicrobial treatment, contribute to its spread. WHO has characterized 14 N. gonorrhoeae strains worldwide. This study aims to determine N. gonorrhoeae strains found among women commercial sex workers in Medan, Indonesia and their potential to antibiotic resistance.

Material and Methods: From 60 women commercial sex workers, it was identified 34 samples, using conventional PCR, positive for N. gonorrhoeae. To confirm that those samples were N. gonorrhoeae, 5 of them were examined for DNA sequencing and similarity to N. gonorrhoeae using blastx.

Results: Samples were identified as N. gonorrhoeae SS3160 strain (n=4) and WHO Y strain (n=1). The naturally occurring cryptic plasmid SS3160 of N. gonorrhoeae is 4,207 base pairs long and is found in about 91% of gonococcal strains while strain WHO Y is 4,153 bp long and is found in about 93%. N. gonorrhoeae strain SS3160 and WHO Y were present in Medan.

Conclusions: WHO Y strain is found, and, therefore possible antibiotic resistance presents in Medan.

Keywords: antibiotic resistance, Neisseria gonorrhoeae, sex workers, strain

I. INTRODUCTION

Neisseria gonorrhoeae is the causative agent of gonorrhoea, one of the most common sexually transmitted infections in the world. Nearly 80 million cases are recorded each year with 45% of those in the Western Pacific region alone (Newman et al., 2015). Women are more often subject to problematic infections. This is because urogenital infection is initially asymptomatic in more than 50% of women and leading to missed opportunities for treatment. Complications may damage the upper genital tract such as pelvic inflammatory disease in women can lead to reproductive problems include infertility (Unemo, Del Rio and Shafer, 2016). High-risk populations for gonorrhoea infection include sexual networks that involved in unprotected sex with multiple partners, particularly commercial sex workers, although recent work suggests that it is the frequency of antimicrobial treatment, not the number of sexual partners, which really contributes to its successful spread (Fingerhuth et al., 2016).

Rates of gonorrhoea vary widely across the world. In Indonesia, the latest Integrated Biological and Behavioral Survey (IBBS) conducted by the Ministry of Health of the Republic of Indonesia in 2013 reported a continuing high prevalence of gonococcal infection, especially among female sex workers (17.7-32.2%) (Ministry of Health Republic of Indonesia – Directorate General of Disease Control and Environmental Health, 2011). However, the IBBS reports lack of important data, such as epidemiology of asymptomatic cases, risk factors for gonorrhoea, and antimicrobial susceptibility. A decreased susceptibility to ceftriaxone has been reported in several centres in Southeast Asia, yet, since 2006, there are no further data available from Indonesia (Bala et al., 2013). This preliminary study aims to identify N. gonorrhoeae strains in Medan and identify their potential to public health threat.
II. RESEARCH METHODOLOGY

*N. gonorrhoeae* isolates. A total of 60 commercial sex workers women were selected for vaginal discharge examination. Using conventional PCR approach and subsequent agarose gel electrophoresis, 34 vaginal discharge samples were identified as positive for *N. gonorrhoeae*. Subsequently, we selected randomly 5 from those 34 samples to examine the *cppB* gene DNA sequence.

*Nucleotide sequencing of *cppB* gene*. Nucleotide sequences from *cppB* gene of positive *N. gonorrhoeae* were run for PCR once again. The primers used in this study are listed in the Table 1 as described before.[6] PCR reaction was carried out in a total volume of 25 µL, with 4 µL of template DNA, 10 pmol of each forward and reverse primers (Ho et al., 1992) and PCR master mix (M7121, Go Taq® PCR Core System, Promega) containing 1 U of Taq polymerase. The thermal cycler was set at 35 cycles of 95 °C for 1 min; 58 °C for 1 min, and 72 °C for 1 min.

The PCR products were sent to a company (Macrogen, South Korea) for subsequent purification and sequencing. All PCR products were sequenced twice with forward and reverse primers using an automatic sequencer. Multiple sequenced alignments of nucleotide and amino acids were performed using ncbi blastx.

III. RESULTS AND DISCUSSIONS

Bacterial strains typing is essential for bacterial infection diagnosis, therapy, and epidemiology. This is particularly critical for bacteria with higher virulence and resistance to antibiotics and those engaged in nosocomial or pandemic diseases. For antimicrobial resistance (AMR) testing, culture and phenotypic AMR testing remain crucial. However, testing small numbers of isolates is laborious and far from ideal for routine AMR testing (Unemo and Shafer, 2014). Examining the genetic makeup of well defined AMR *N. gonorrhoeae* may become an diagnostic tool and therapy approach.

Current bacterial genotyping techniques are divided into three primary classifications: (1) DNA banding pattern-based approaches classifying bacteria by the size of fragments produced by amplification and/or enzymatic digestion of genomic DNA, (2) DNA sequencing-based techniques studying DNA sequence polymorphism, and (3) DNA hybridization-based technique (Li, Raoult and Fournier, 2009). DNA banding pattern-based genotyping techniques discriminated against the species studied based on variations in the size of the DNA bands produced by genomic DNA amplification or DNA cleavage using restriction enzymes. DNA sequencing-based genotyping techniques produce the initial nucleotide sequence and discriminate directly from polymorphisms in their DNA between bacterial species. Methods based on DNA hybridization are primarily referred to as research of DNA macroarray and microarray. Bacterial strains are discriminated against in this method by evaluating their DNA hybridization to probes of known sequences. The discriminatory capacity of genotyping techniques is dependent on species, with the exception of genome sequencing (Li, Raoult and Fournier, 2009).

This study conducted DNA sequencing-based genotyping techniques in order to determine *N. gonorrhoea* strains from 5 commercial sex workers. Samples examined were identified as *N. gonorrhoeae* SS3160 strain (n=4) (Figure 1) and WHO Y strain (n=1) (Figure 2). The naturally occurring cryptic plasmid SS3160 of *N. gonorrhoeae* is 4,207 base pairs long and is found in about 91% of gonococcal strains while WHO Y strain is 4,153 bp long and is found in about 93%. Therefore, *N. gonorrhoeae* SS3160 and WHO Y strains were present in Medan.

Another problem with gonococcal infection was disseminated gonococcal infection. This infection has potentially causes for many complications, and the pathogen has recently been reported to be resistant to various antibiotics (Hagiya et al., 2013). The emergence and dissemination of the ceftriaxone-resistant strains are serious clinical problems. *N. gonorrhoeae* SS3160 strain were reported as ceftriaxone-resistant bacteria. The acquisition of ceftriaxone resistance by *N. gonorrhoeae* strains is typically attributed to a mosaic mutation in the penicillin-binding protein encoded by penA (Miyake et al., 2020). In 2016, *N. gonorrhoeae* WHO Y strain was classified as 1 of 14 reference strains for global quality assurance of laboratory investigations. It is also showed that this strain has high-level resistant to cefixime, ceftriaxone, and ciprofloxacin (Unemo et al., 2016). The presence of both AMR *N. gonorrhoeae* strains has threaten the public health.

In Indonesia, spreading of *N. gonorrhoeae* infections are traditionally identified by contact tracing using self-reported sexual history from STI clinics. However, this approach may inform recall bias, socially desirable responses, and privacy issues. The use of molecular typing techniques may improve the quality of transmission network identification by providing information on the spread of bacterial strains in different populations. Multiple-
Locus Variable Number Tandem Repeat Analysis (MLVA) is a fast and robust method for molecular typing of *N. gonorrhoeae* strains which is based on the variation in the number of tandem repeated DNA sequences in five loci of the bacterial genome. *N. gonorrhoeae* Multi-Antigen Sequence Typing (NG-MAST) is a method using the combination of allele sequence variations of two gonococcal genes, *porB* and *tbpB*, to determine the strain type (Unemo and Dillon, 2011).

Using Multiple-Locus Variable Number Tandem Repeat (VNTR) Analysis (MLVA) and Neisseria gonorrhoeae Multi-Antigen Sequence Typing (NG-MAST), Hananta et al. (2018) reported that the most prevalent NG-MAST genotype groups among Indonesian strains was G1407 (51.3%), the AW [95% confidence interval] for MLVA to NG-MAST was 0.07 [0.00–0.27] and for NG-MAST to MLVA was 0.03 [0.00–0.12] (Hananta et al., 2018).

A bacterial strain is made up of the descendants of a single isolation in pure culture and usually is made up of a succession of cultures ultimately derived from an initial single colony described in 1st Bergey’s Manual of Systematic Bacteriology. This referred to the strain in the taxonomic sense. Moreover, there is a counterpart in nature to the strain in taxonomic sense and simply refer to it as the strain in nature. Both bacterial strains undergo mutations and they may lose plasmids (Dijkshoorn, Ursing and Ursing, 2000).

The ability to study and compare genomes as well as progress of molecular biology has promised an insight into evolutionary relationships and a classification based on natural affinity. This has led to show that genotypic data are the most important. A side effect of this progress showed that taxonomic work has been increasingly undertaken by biochemists rather than microbiologists because it is easier to identify chemical compounds than to classify than organisms (Dijkshoorn, Ursing and Ursing, 2000).

Several plasmids were found in gonococcal clinical isolates. About 96% isolates possess this cryptic plasmid. The smallest of these plasmids is about 4.2 kilobases (kb) in size, the *cppA* gene encodes a 9 kDa protein, and the *cppB* and *cppC* genes both code for 24 kDa proteins. However, their functions were unknown (Korch *et al.*, 1985). Amino acid sequences of *cppB* gene from the *N. gonorrhoeae* clinical isolates examined in this study shows the sequence of cryptic plasmid protein B (Figure 3). Amino acid sequences of genome from the *N. gonorrhoeae* clinical isolates examined in this study shows sequence of putative relaxase/mobilization nuclease MobA (Figure 4). WHO Y strain is one of five *N. gonorrhoeae* strains which contain *penA* allele (main Extended Spectrum Cephalosporin (ESC) resistance determinant). WHO Y also contained a penicillin-binding protein 2 (PBP2) A501V and A501P alteration, respectively, which also increase the minimum inhibitory concentration of beta-lactam antimicrobials including ESCs (Unemo *et al.*, 2016). The putative relaxase/mobilization nuclease MobA function has not been determined yet.

**IV. CONCLUSION**

We reported that in Medan, AMR *N. gonorrhoeae* SS3160 and WHO Y strains were found and the spread of these *N. gonorrhoeae* strains with reduced susceptibility to extended-spectrum cephalosporins should increase alarm for public health threat.

**Limitation and Study Forward**

This preliminary result will be followed by further works with identification of the positive samples for the genetic makeup of cefixime- and ceftriaxone-resistance *N. gonorrhoeae* using conventional PCR, sequencing and blastx.

**Acknowledgement**

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REFERENCES


Table 1. Primers used for PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence (5' to 3')</th>
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<tr>
<td>Forward</td>
<td>GCTACGCATACCCGCGT1TGCC</td>
</tr>
<tr>
<td>Reverse</td>
<td>CGAAGACG1TCCAGCAGCACAGA</td>
</tr>
</tbody>
</table>

Query 9

CGACCCGGGC-ATTTCCTCAGTGTCCAACCTTTTGGTTTCTCTTTCCACAGGSTTCTA 67

Sbjct 3011

CGACTGGGCAATTTTCCAG-TGCTCAGTCTTTCTCAGTCTCGAGGCTTCTGACGCT 127

Query 256

GCTACGCATACCCGCGT1TGCC

Sbjct 1275

CGAAGACG1TCCAGCAGCACAGA

Query 367

AGGTCTTCG 375

Sbjct 2659

GGTCTTCG 2652

Figure 1. Alignment of N. gonorrhoeae cppB gene sequence strain SS3160 (GenBank accession number AP019855.1) isolates from this study

Query 12

GCC-GCATAGAGC-ACCAACGAGCAGAATTTAGACGTTCGAGAAAATATCAAC 69

Sbjct 3442

GCCAGCATAGAGCAACAAACGAGCAGAATTTAGACGTTCGAGAAAATATCAAC 3386

Query 70

GAAGGCATTGAAGCAAAGCGAGCAGAAAATAACCGCCGATATAAACGCTCGGCAGTTACG 129

Sbjct 3385

GA-GCATAGAGCAGAATTTTAGACGTTCGAGAAAATATCAAC 3327

Query 130

CATGAGCAAGCAGTATTCAACCCCTATCTGTGGAGCTTGCTAGGTATATCGGCGGCAGG 189

Sbjct 3326

CATGAGCAAGCAGTATTCAACCCCTATCTGTGGAGCTTGCTAGGTATATCGGCGGCAGG 3267

Query 190

CTTGATAGTCATAGCAGGGCTGTTCATAGCGATATGGAGCGTCAAGAACGAGCTGGACGA 249

Sbjct 3266

CTTGATAGTCATAGCAGGGCTGTTCATAGCGATATGGAGCGTCAAGAACGAGCTGGACGA 3207

Query 250

CTTGAAACAGCAGGAGCCGAGACGAGCAGACCCCTAGACCTGTTGGAAACCAAGACCAA 309

Sbjct 3206

CTTGAAACAGCAGGAGCCGAGACGAGCAGACCCCTAGACCTGTTGGAAACCAAGACCAA 3147

Query 310

AGGTTGACACTGGAACATTCGCCAGTAGCTCAGAGAAGCAGAAGCAGG 358

Sbjct 3146

AGGTTGACACTGGAACATTCGCCAGTAGCTCAGAGAAGCAGAAGCAGG 3098

Figure 2. Alignment of N. gonorrhoeae genome sequence strain WHO Y (GenBank accession number LT592162.1) isolates from this study
Figure 3. Amino acid sequences of cppB gene from the N. gonorrhoeae clinical isolates examined in this study

Figure 4. Amino acid sequences of N. gonorrhoeae genome sequence strain WHO Y clinical isolates examined in this study