**Validation of *sodC* gene-based PCR assay and antimicrobial resistance profiling of *Neisseria* *meningitidis* in asymptomatic carriers**

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**Abstract**

**Background:** *Neisseria meningitidis* is one of the leading causes of bacterial meningitis and septicemia worldwide. The bacteriological culture was a widely used method for the detection of meningococcus, but it has low sensitivity and long waiting periods. Molecular detection targeting capsule transport gene was used, but over 16% of meningococcal carriage isolates lack *ctrA* and generate false-negative results due to sequence variations. The Cu-Zn superoxide dismutase gene (*sodC*) is specific to *N. meningitidis*, not found in other *Neisseria species*, making it better able to identify encapsulated meningococci and useful for detecting non-group-able meningococci without intact *ctrA*.

**Objective:** The objective of this study was the validation of *sodC* gene-based PCR assay and antimicrobial resistance profiling of *N. meningitidis* in asymptomatic carriers

**Methods: The** *sodC* gene *N. meningitidis* detection method was developed using a pair of primers and optimized. A total of 137 archived samples that were collected from the asymptomatic carrier suspected of having meningococcal infection were used for validation of the assay. STATA version 14.0 was used for analysis of clinical and demographic data after the data was entered into Epi Info version 7. Graphs and frequency tables from descriptive statistics were used to summarize the outcome. Two-by-two tables were used to compare the sensitivity specifically between the *sodC*-based PCR assay and culture and *ctrA*-based PCR. A Disk diffusion test was used to determine the antimicrobial sensitivity of the isolates against antimicrobial drugs. To determine the association between independent and outcome variables, bivariate and multivariate logistic regression models were used and P-values less than 0.05 were considered statistically significant.

**Result:** The PCR assay targeting the *sodC* gene detected *N. meningitidis* DNA in 105 (76.6%) out of 137 clinical samples, while *ctrA*-based PCR detected the pathogen in 64 (46.7%) of the samples, and 49 samples (35.8%) of *N. meningitidis* were identified by culture. Then, the concordance of our in-house PCR assay targeting the *sodC* gene with *ctrA* PCR was performed using 137 clinical samples (Nasopharyngeal swabs). Among the 49 DNA samples from culture-positive *N. meningitidis* isolates used for validation, the *sodC* gene-based PCR accurately identified all 49 culture-confirmed isolates. In contrast, the *ctrA* gene-based PCR detected only 33 of these isolates. Out of the 49 *N. meningitidis* isolates by culture 43 (87.8%), 42 (83.7%), 32 (65.3%), 22 (44.9%), and 18 (36.7%), and 7 (15.2%) were resistant to amoxicillin, ampicillin, trimethoprim-sulfamethoxazole, ceftazidime, ceftriaxone, and meropenem, respectively. Furthermore, the majority of *N. meningitidis* isolates 36 (73.5%) were sensitive to cefepime, 31 (63.3%) of them were sensitive to ceftriaxone and meropenem, and 26 (53.1%) of them were sensitive to ceftazidime.

**Conclusion**: The *sodC* gene-based PCR assay demonstrated high sensitivity in detecting *N. meningitidis* in carriage specimens compared to *ctrA* gene-based PCR. The high prevalence of antibiotic resistance observed is alarming and highlights the urgent need to continue monitoring antibiotic resistance to inform treatment strategies effectively.

**Keywords:** PCR, Molecular diagnostics**,** *Neisseria meningitidis*, *sodC*, *ctrA*, Antimicrobial resistance, Ethiopia

**Introduction**

*Neisseria meningitidis*, often referred to as meningococcus, is a gram-negative bacterium that can cause a spectrum of diseases ranging from mild sepsis with rapid recovery to fulminant meningococcemia (1). They are carried by healthy individuals in the nasopharynx around 5-10% (2). Every year, more than 1.2 million cases of bacterial meningitis are estimated to occur globally, causing about 14.25% of deaths (3). In Ethiopia, bacterial meningitis is an important cause of premature death and disability, being the 9th most common cause of years of life lost and disability-adjusted life years. Early treatment is essential in the clinical management of meningitis. A delay in therapy negatively affects the prognosis for patients with meningitis (4). The incidence of meningitis varies depending on age, geographic location, species, genotype, strain, and serotype of the causative agents (5, 6).

Accurate diagnosis and early treatment of meningococcal infections are essential due to their worldwide distribution, increased case fatality and morbidity rate, epidemic potential, and serious complications that can occur (7). For confirming the etiology, CSF and/or blood culture have been used as the gold standard for the diagnosis of meningococcal infection (8). Identification and detection of bacterial pathogens in cases of suspected meningitis are important in guiding appropriate treatment and prophylaxis. However, diagnosis of bacterial meningitis is often difficult (9). Traditional laboratory diagnosis of meningococcal disease (MD) has relied heavily on bacteriologic culture methods, but the bacterial growth rates, particularly in patients who have received pre-admission antibiotic treatment, are very low and have low sensitivity due to the frequent initiation of antimicrobial therapy before clinical sample collection (10). Many studies have shown that the high rates of morbidity and mortality linked to MD, particularly in children, are partly caused by delayed detection and diagnosis (11-14).

Molecular assays can be used for the diagnosis of invasive meningococcal infections when previous antibiotic therapy may inhibit bacterial growth (8). In previous reports, either *ctrA* or *sodC* genes were independently used for detecting meningococcal DNA, which has different sensitivities, both of which are superior to culture (15-18). The genome of *N. meningitidis* displays extensive genetic variability within a single course of infection by genome rearrangement (19). The high genetic diversity of *N. meningitidis* makes reliable laboratory detection of this important pathogen difficult (20-22). A *ctrA* was thought to be found in all invasive strains of *N. meningitidis* due to the importance of the capsule in preventing complement-mediated killing (23). However, there has been a question raised about the sensitivity of *ctrA* in the identification of invasive *N. meningitidis* isolates. Several cases of invasive and sometimes fatal disease caused by capsule-null (cnl) strains lacking *ctrA* have been described (24-27).

Given that *ctrA* is not present in 16% or more of carriage isolates, it is not a suitable target gene for PCR on carriage study specimens. The reliability of *ctrA*-based PCR assays in detecting non-groupable invasive *N. meningitidis* isolates or specimens containing them is in question chiefly due to the genetics of non-groupable *N. meningitidis* carriage isolates and their genome plasticity. Because of natural competence, frequent horizontal gene transfer, and high rates of recombination, *N. meningitidis* has a significant degree of genomic diversity, which makes it difficult to utilize a single gene for identification (16, 28, 29). Also, *ctrA*-based molecular assay has been reported to generate false-negative results due to sequence variations in *ctrA* (15, 28, 30). Non-capsular genes like *sodC*, *metA*, and *tauE* provide complementary targets to *ctrA* to improve the detection of *N. meningitidis* via multiplex PCR (31).

Early antibiotic treatment of meningococcal disease is crucial for keeping the case fatality rate and risk of sequelae as low as possible (32). Resistance to various antimicrobial drugs used either for therapy of invasive infections or for prophylaxis of case contacts has long been recognized. There is limited data on the antimicrobial susceptibility test (AST) pattern of *N. meningitidis* in Ethiopia in general (27, 33).

The development of reliable molecular approaches for the detection and characterization of *N. meningitidis* is clinically relevant. Molecular methods offer several advantages over culture-based methods, including increased sensitivity, specificity, speed, and efficiency. Furthermore, generating information about the AST pattern of *N. meningitidis* is crucial to identify resistance in the meningococcus to prevent treatment failure because of the administration of inappropriate antibiotics (34).

Thus, the aims of this study were validation of *sodC* gene-based PCR assay and antimicrobial susceptibility pattern of *N. meningitidis* isolates.

**MATERIALS AND METHODS**

**Ethical consideration**

This study was done on clinical (pharyngeal swabs) and culture samples that were collected from a MenAfricar study in 2016 that employed a cross-sectional study and stored in the AHRI. Ethical waiver to use those archived isolates and clinical samples from All African Lepersoy Rehabilitation Center (ALERT)/ Armauer Hansen Research Institute (AHRI) Ethics Committee with approval number: PO-63-22.

**Study Isolates and Clinical Samples**

In 2016, respiratory clinical specimens were collected from 3000 asymptomatic *Neisseria* spp carriers. The leftover specimens (pharyngeal swabs) and culture-positive, 49 isolates, were stored at -80°C in 1 ml of STGG medium (containing Skim milk, Tryptisoya, Glucose, and Glycerol) at the AHRI microbiology laboratory. To assess its performance in clinical specimens, 137 archived specimens preserved in 1 ml of STGG were randomly selected and the *sodC* gene-based PCR assay was validated. Demographically of the total clinical samples, 71 (52%) were collected from males and 66 (48%) were from females. The age categories of 15–19 had the highest percentage (26%) while the age groups of 1-4 had the lowest (6%). The age group ranges from 1 to 29 years, with a mean age of 16.38 years (Table 1).

**Characterization and confirmation of *N. meningitidis isolates***

Cryopreserved bacterial isolates were thawed at room temperature. A loopful of these samples was then sub-cultured onto fresh chocolate (CHO) and modified Thayer Martin (MTM) agar plates, which were supplemented with vancomycin, colistin, nystatin, trimethoprim (VCNT), and IsoVitaleX enrichment. Colonies were further confirmed using gram stain and oxidase tests. After confirming the presence of gram-negative diplococci and oxidase-positive isolates, a pure colony was sub-cultured again on a blood agar plate (BAP) with 5-10% CO2 for 24 to 48 hours to ensure the purity of the culture for further biochemical testing.

A carbohydrate utilization test (glucose, maltose, lactose, and sucrose) was performed using cystine trypticase agar (CTA) to differentiate *N. meningitidis* from Moraxella species and other nonpathogenic *Neisseria* species. Isolates that were gram-negative diplococci, oxidase-positive, glucose fermenters, maltose fermenters, and non-fermenters of lactose and sucrose were confirmed as *N. meningitidis,* a total of 49 isolates.

**Antimicrobial susceptibility testing (AST)**

AST was performed on 49 isolates of *N. meningitidis*, all of which exhibited the standard characteristics of *N. meningitidis* colonies using Mueller-Hinton agar (MHA) with 5% sheep blood (34). Briefly, 3-5 colonies from a blood agar plate were suspended, and the turbidity was adjusted to match a 0.5 McFarland standard. The surface of the MHA with 5% sheep blood agar was then thoroughly coated with the bacterial suspension using a sterile swab. After allowing the plate to dry for 3-5 minutes, antibiotic discs were evenly placed on the inoculated plate with sterile forceps. The plate was incubated in 5% CO2 at 37ºC for 24 hours. The antibiotics selected for AST profiling were based on the Ministry of Health Ethiopia guidelines, fourth edition 2021(<https://www.slideshare.net/TesfayeWorkie/stg-2021pdf#1>). The tested antibiotics included Ceftriaxone (30μg), Meropenem (30µg), Cefepime (30µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), Ampicillin (10µg), Amoxicillin (10µg), and Ceftazidime (30µg). The diameters of the inhibition zones around the discs were measured to the nearest millimeter using a graduated caliper, and these measurements were compared to standard charts to determine the susceptibility, intermediate resistance, or resistance of the bacteria to the tested antibiotics, according to clinical and laboratory standard institute 2022.

**DNA Preparation and Quantification**

DNA was isolated from 137 clinical samples (pharyngeal swabs) preserved in STGG broth, as well as from the 49 isolates. Briefly, samples stored in 1 ml of STGG broth at -80ºC were thawed at room temperature. Then, 200 μl of the sample was mixed with 200 μl of 1x TE buffer; in the case of the cultured sample, 3-5 colonies were picked up from the agar plate, then vortexed vigorously, and incubated in a water bath at 95ºC for 15 minutes. The tubes were then transferred to -20ºC for 10 minutes to release bacterial DNA through heat shock, followed by a 2-minute incubation at room temperature. The tubes were centrifuged at 14,000 rpm for 5 minutes, and the genomic DNA remained in the upper aqueous phase (supernatant). The supernatant containing the DNA was transferred to a separate 2 ml sterile tube, and the DNA concentration was measured using a NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, USA). The DNA was then stored at -80ºC until used as a template for the PCR assay.

**In-house Development of *sodC*-based PCR assay**

We selected the *sodC* gene as the target for developing a PCR assay aimed at detecting *N. meningitidis in* clinical samples. The *sodC* gene was chosen for several reasons: (i) it offers high specificity for detecting *N. meningitidis* as it is absent in other *Neisseria* species; (ii) the *sodC* gene in *N. meningitidis* encodes virulence factors and a periplasmic enzyme, making it less prone to antigenic variation due to selective pressure and (iii) there are no known strains of meningococci that lack the *sodC* gene. These factors collectively suggest that a PCR assay based on the *sodC* gene can detect all *N. meningitidis* strains from various geographical regions without cross-reacting with other *Neisseria species* (15).

***Primer design***

Primers (both forward and reverse) were designed using SnapGene Viewer (Table 2). The *sodC* sequences of *N. meningitidis* were sourced from Gene Bank (accession number >NZ\_CP021520.1:999157-999717). The specificity of these primers to *N. meningitidis* was verified using the NCBI nucleotide BLAST tool.

***sodC* PCR amplification conditions**

The *sodC* PCR reaction was performed in a 25 μl mixture, which included 0.625 μl of each primer (10 μM), 2.5 μl of dNTPs (2.5 μM), 0.5 μl of DNA polymerase, 2.5 μl of polymerase buffer with magnesium acetate (5 μl of 10x), 15.25 μl of nuclease-free water, and 3 μl of genomic DNA as the template. Each run incorporated both positive and negative controls. The optimized amplification protocol started with an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 10 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. The amplified product was then analyzed on a 2% agarose gel using 1x Tris-acetate EDTA (TAE) buffer and visualized with Bio-Rad UV scanning (a chemi PRO UV scanner, USA). The optimal conditions for the *sodC* PCR reaction and the most effective primer pairs were identified. This assay was then validated using the culture technique as the gold standard and then compared with the *ctrA* PCR assay.

***ctrA* gene-based PCR assay**

The *ctrA* gene-based PCR assay, which includes the paired oligonucleotide primer (Table 3), was initially developed by Lansac N, et.a1 in 2000 (35), and later used at AHRI (36). Briefly, the PCR reactions comprised: 0.625 μl of both forward and reverse primers (10 μM each), 2.5 μl of dNTPs (2.5 μM), 0.5 μl of DNA polymerase, 2.5 μl of polymerase buffer with magnesium acetate (5 μl of 10x), 15.25 μl of nuclease-free water, and 3 μl of genomic DNA as the template, making a final volume of 25 μl. Each run included positive and negative controls. The amplification protocol was optimized with an initial denaturation at 95°C for 3 minutes, followed by 30 seconds at 94°C for denaturation, 45 seconds at 52°C for annealing, and 1 minute at 72°C for extension, with a final extension at 72°C for 10 minutes over 35 cycles. The amplified product was analyzed on a 2% agarose gel using 1x TAE buffer and visualized with Bio-Rad UV scanning (a chemi PRO UV scanner, USA). Representative gel images of the PCR products from both the *sodC* and *ctrA* genes, obtained from control strains ATCC (serogroup A: Z2491; W: A22; X: 860060; Y: 71/94) and suspected samples, are shown in Figure 2.

**Data Management and Analysis**

The data obtained was entered into Epi Info version 7 and exported into STATA version 14 for data cleaning and analysis. Results were presented in frequency tables and charts, descriptively. Bivariate and multivariate logistic regression models were used to analyze data and P-values less than 0.05 were considered statistically significant.

**Results**

***Optimal conditions for the sodC gene-based PCR assay***

To determine the optimal conditions for sodC gene-based PCR assay, a set of primer pairs targeting *sodC* gene was optimized by using the temperature gradient of the annealing temperature (Figure 1) and concentration of oligonucleotide forward and reverse primer pairs (Table 2). The *sodC* gene target was amplified using a selected optimized concentration of pair of primers at optimized PCR conditions: initial denaturation at 95°C for 3 min, followed by 35 cycles, denaturation at 94°C for 10 sec, annealing at 56°C for 30 sec, extension at 72°C for 30 sec, and a final extension at 72°C for 10 min.

***Performance comparison between sodC* gene-based *detection of N. meningitidis and ctrA*-based detection**

To validate our in-house developed *sodC* gene-based assay for detecting *N. meningitidis*, we utilized culture-positive *N. meningitidis* isolates and compared the results with those from the *ctrA*-based PCR detection method. Among the 49 DNA samples from culture-positive *N. meningitidis* isolates used for validation, the *sodC* gene-based PCR accurately identified all 49 culture-confirmed isolates. In contrast, the *ctrA* gene-based PCR detected only 33 of these isolates. This demonstrates a 100% concordance between the culture method and the *sodC* gene-based PCR assay (Table 4). After validating our in-house PCR assay targeting the *sodC* gene with culture-positive isolates, we assessed its concordance with the PCR assay targeting the *ctrA* gene using 137 clinical pharyngeal swab samples. The *sodC* gene-based method detected *N. meningitidis* DNA in 76.64% of the samples, whereas the *ctrA* gene-based method identified it in only 46.72% (Table 5).

The bivariate and multivariate analyses revealed that variables such as sex, age, and residence of the study participants did not have a significant impact on the detection of *N. meningitidis* using the *sodC* gene-based PCR method(Table 6).

**Antimicrobial susceptibility pattern of *N. meningitidis* isolates**

The antimicrobial susceptibility patterns of the 49 *N. meningitidis* isolates are summarized in Table 7. Among these isolates, resistance was found in 43 (87.8%) to amoxicillin, 42 (83.7%) to ampicillin, 32 (65.3%) to trimethoprim-sulfamethoxazole, 22 (44.9%) to ceftazidime, and 18 (36.7%) to both ceftriaxone and meropenem. Additionally, 7 isolates (15.2%) showed resistance to cefepime. On the other hand, a notable number of isolates were sensitive to cefepime (36 isolates, 73.5%), ceftriaxone and meropenem (31 isolates, 63.3%), and ceftazidime (26 isolates, 53.1%).

**Discussion**

The results of this study offer important insights into the diagnostic techniques for the detection of *N. meningitidis*, particularly focusing on comparing PCR-targeted *sodC* and *ctrA* genes. The findings clearly show that, compared to the *ctrA* gene, the *sodC-based* PCR is a more sensitive and accurate method to detect *N. meningitidis*.

The higher detection rate of *N. meningitidis* using PCR targeting the *sodC* gene highlights the potential of this method as an effective tool for the diagnosis of meningococcal disease (37). In contrast, the lower detection rates of *N. meningitidis* using the *ctrA* gene methods suggest that this method may be less sensitive and reliable for detecting bacteria in clinical samples. This has important implications for clinical diagnosis, as timely and accurate detection of *N. meningitidis* is essential for initiating appropriate treatment and implementing public health measures to prevent the spread of the disease. A similar study that tested pharyngeal swabs for the presence of *N. meningitidis* by PCR with *sodC* and *ctrA* as target genes identified 75.8% (491/647) of clinical samples were tested positive for the *sodC* gene (38).

Furthermore, *sodC*-based real-time PCR identified a higher detection rate of *N. meningitidis isolates,* 518 of 520 (99.6%) isolates of *N. meningitidis* were positive for *sodC*, whereas *ctrA* detection occurs only in 368 of 520 (70.8%) isolates. Similar to our report, *sodC* gene-based PCR shows higher sensitivity than the *ctrA* gene (15).

Contrary to our finding, the same molecular assay for the detection of meningococci in normally sterile sites *sodC* and *ctrA* genes was used, and *sodC*-based RT-PCR was found less sensitive by 7.5% than *ctrA* (16).

Resistance to trimethoprim-sulphamethoxazole was high among meningococcal isolates. The widespread resistance to this antibiotic is possibly due to the early introduction of sulphonamides (39). In contrast, an investigation in children in Greece in 2004 revealed that ceftriaxone sensitivity was present in all isolates (40). There was no significant effect of the study participant's sex and age on the AST pattern of *N. meningitidis* (Table 8 and 9 respectively). However, we noted that the percentile of antibiotic resistance of *N. meningitidis* to different drugs differed slightly based on the age group; for example, those aged 24-29 showed the lowest percentile of resistance to ampicillin and amoxicillin, while *N. meningitidis* isolates fromthose aged between 10 and 14 showed the highest percentile of resistance to those of the same antimicrobial drugs.

*N. meningitidis* is developing a resistance to antibiotics that are recommended for the management of meningococcal meningitis for epidemic response. These findings have important implications for clinical practice and public health. Healthcare providers should be aware of the drug susceptibility profile of *N. meningitidis* in their region and consider these factors when selecting antimicrobial therapy for patients with suspected or confirmed meningococcal infections. Additionally, public health efforts should focus on surveillance of antimicrobial resistance in *N. meningitidis* and the development of new treatment strategies to combat resistant strains. Furthermore, the ability of PCR targeting the *sodC* gene to detect a larger proportion of *N. meningitidis* isolates has implications for epidemiological studies and surveillance of meningococcal disease. Accurate and comprehensive surveillance data are essential for understanding the epidemiology and dynamics of *N. meningitidis* transmission, as well as for informing public health interventions such as vaccination strategies. The higher detection rate of *N. meningitidis* using PCR targeting the *sodC* gene may also be valuable for identifying asymptomatic carriers of the bacteria, which is critical for implementing targeted control measures to prevent outbreaks.

**Conclusion**

The *sodC* gene-based PCR assay is found to be superior in sensitivity to detecting *N. meningitidis* in carriage specimens compared to *ctrA* gene-based PCR. The observed high prevalence of antibiotic resistance warrants the need to continue to monitor antibiotic resistance that might influence treatment and for future implementation of chemoprophylaxis for carriers and those household members who have contacts with confirmed meningitis cases.

**Limitations of the study**

The study was conducted in a limited number of samples archived in the laboratory.

**Abbreviations**

AHRI, Armauer Hansen Research Institute; AST, Antimicrobial susceptibility testing; ATCC, American Type Culture Collection; CSF, Cerebrospinal fluid; *ctrA*, Capsule transport to cell surface gene; DNA, Deoxyribonucleic acid; MD, Meningococcal disease; MHA, Muller Hinton Agar; PCR, Polymerase chain reaction; *sodC*, Cu-Zn superoxide dismutase gene

**Data Sharing Statement**

The data supporting the findings of this study are available upon reasonable request from the corresponding author.

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**Disclosure**

The authors declare no conflicts of interest.

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Table 1: Socio-demographic characteristics for the asymptomatic nasopharyngeal carriage rate of *N. meningitidis*

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics of the study population**  **(n=137)** | | **Number** | **Percentage** |
| Sex | Male | 71 | 52 |
| Female | 66 | 48 |
| Residence | Urban | 58 | 42.34 |
| Rural | 79 | 57.66 |
| Age | 1-4 | 8 | 6 |
| 5-9 | 15 | 11 |
| 10-14 | 33 | 24 |
| 15-19 | 36 | 26 |
| 20-24 | 31 | 23 |
| 25-29 | 14 | 10 |

Table 2: List of Oligonucleotide primers designed for the *N. meningitidis* *sodC* gene target

|  |  |  |
| --- | --- | --- |
| S. no | Oligonucleotide | 5’-3’ nucleotide sequences |
| 1. | *sodC* Fw1-PCR | ATGAATATGAAAACCTTATTAGCACTAGCGGTTAGTGCAG |
| 2. | *sodC* Fw14-48 | CCTTATTAGCACTAGCGGTTAGTGCAGTATGTTC |
| 3. | *sodC* Fw14-PCR | CCTTATTAGCACTAGCGGTTAG |
| 4. | *sodC* Fw64 | GCACACGAGCATAATACGATACCTAAAGGTGCTTC |
| 5. | *sodC* Fw118 | CAACTTGATCCAGCAAACGGTAACAAAGATGTGGG |
| 6. | *sodC* Fw361 | GCACACTTAGGTGATTTACCTGCATTAACTG |
| 7. | *sodC* Rv478-PCR | GGATCATAATAGAGTGACCGCGAAC |
| 8. | *sodC* Rv520-PCR | CAAGTGGAGCTGGATGATCGGAGTG |
| 9. | *sodC* Rv561-PCR | TTATTTAATCACGCCACATGCCATACGTGG |

Table 3: Oligonucleotide primers used in this study for the *ctrA* gene target

|  |  |  |  |
| --- | --- | --- | --- |
| Gene and group | Designation | 5’-3’ nucleotide sequences | Bp |
| CtrA | *ctrA*-F | GCTGCGGTAGGTGGTTCAA | 110 |
| *ctrA*-R | TTGTCGCGGATTTGCAACTA |

Table 4: Comparative concordance of the two molecular detection techniques on 49 *N. meningitidis* isolates confirmed by culture

|  |  |  |  |
| --- | --- | --- | --- |
|  | Positive | Negative | Total |
| *sodC* | 49 | 0 | 49 |
| *ctrA* | 33 | 16 | 49 |

Table 5: Comparison of the *sodC* gene-based PCR assay and the *ctrA* gene-based PCR assay for detecting *N. meningitidis* in 137 DNA samples derived from 137 clinical specimens

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | N (DNA samples) | Positive | Negative | Total |
| *sodC* | 137 | 105 | 32 | 137 |
| *ctrA* | 137 | 64 | 73 | 137 |

Table 6: Bivariable and multivariable logistic regression analysis for independent variables associated with *N. meningitidis* DNA detected using *sodC* gene-based PCR

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Independent Variables | *SodC* gene-based PCR | | OR (95% CI) | p-value | AOR (95% CI) | p-value |
| Positive | Negative |
| Sex  Male  Female | 37  15 | 195  209 | 1.1 (0.5, 2.4)  1.0 | 0.81 | 1.04 (0.47, 2.32)  1.0 | 0.93 |
| Age  ≥21  11-20  <10 | 29  57  19 | 9  15  8 | 1.4 (0.45, 4.13)  1.6 (0.59, 4.36)  1.0 | 0.59  0.36 | 1.32 (0.43, 4.05)  1.52 (0.55, 4.19)  1.0 | 0.63  0.42 |
| Resident  Urban  Rural | 43  62 | 10  22 | 1.5 (0.65, 3.5)  1.0 | 0.33 | 1.47 (0.63, 3.44)  1.0 | 0.11 |

Table 7: Drug susceptibility profile of 49 *N. meningitidis* isolates

|  |  |  |  |
| --- | --- | --- | --- |
| Antimicrobials with Disk content |  | No (49) | % |
| Ceftriaxone /CRO -30µg | I | - | - |
| R | 18 | 36.7 |
| S | 31 | 63.3 |
| Ampicillin(10µg), | I | - | - |
| R | 42 | 83.7 |
| S | 8 | 16.3 |
| Amoxicillin (10µg), | I | - | - |
| R | 43 | 87.8 |
| S | 6 | 12.2 |
| Trimethoprim  sulfamethoxazole1/SXT  1.25/23.75µg | I | 6 | 12.2 |
| R | 32 | 65.3 |
| S | 11 | 22.5 |
| Cefepime(30µg) | I | 3 | 11.3 |
| R | 7 | 15.2 |
| S | 36 | 73.5 |
| Meropenem(30µg) | I | - | - |
| R | 18 | 36.7 |
| S | 31 | 63.3 |
| Ceftazidime(30µg) | I | 1 | 2 |
| R | 22 | 44.9 |
| S | 26 | 53.1 |

Table 8: Bivariable logistic regression analysis for association between sex and antibiotic susceptibility pattern of 49 *N. meningitidis* isolates

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antibiotics** | | **Sex** | | **OR (95% CI)** | **P-value** |
| Male (n=26) | Female (n=23) |
| Ceftriaxone | S | 18 | 13 | 1.73 (0.54, 5.58) | 0.36 |
| R | 8 | 10 |
| Ampicillin | S | 5 | 3 | 1.58 (0.33, 7.53) | 0.56 |
| R | 21 | 20 |
| Amoxicillin | S | 3 | 3 | 0.87 (0.15, 4.80) | 0.87 |
| R | 23 | 20 |
| Trimethoprim sulfamethoxazole/SXT | S | 6 | 5 | 1.08 (0.28, 4.15) | 0.91 |
| R | 20 | 18 |
| Cefepime | S | 20 | 19 | 0.70 (0.17, 2.88) | 0.62 |
| R | 6 | 4 |
| Meropenem | S | 18 | 13 | 1.73 (0.54, 5.59) | 0.36 |
| R | 8 | 10 |
| Ceftazidime | S | 15 | 11 | 1.49 (0.48, 4.60) | 0.49 |
| R | 11 | 12 |

Table 9: Bivariable logistic regression analysis for the association between age and antibiotics susceptibility pattern of 49 *N. meningitidis* isolates

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Antibiotics** | | **Age** | | | **OR (95% CI)** | **P-value** |
| <10 | 11-20 | ≥21 |
| Ceftriaxone | S | 5 | 16 | 10 | 1.00  2.0 (0.54, 5.59)  1.28 (0.54, 5.59) | 0.44  0.75 |
| R | 4 | 10 | 4 |
| Ampicillin | S | 2 | 4 | 2 | 1.00  0.64 (0.09, 4.24)  0.58 (0.06, 5.11) | 0.64  0.63 |
| R | 7 | 22 | 12 |
| Amoxicillin | S | 1 | 3 | 2 | 1.00  1.04 (0.09, 11.52)  1.33 (0.10, 17.27) | 0.97  0.82 |
| R | 8 | 23 | 12 |
| Trimethoprim-sulfamethoxazole/SXT | S | 2 | 6 | 3 | 1.00  0.95 (0.12, 7.23)  1.05 (0.17, 6.46) | 0.96  0.95 |
| R | 7 | 20 | 11 |
| Cefepime | S | 8 | 19 | 12 | 1.00  0.75 (0.06, 9.72)  0.34 (0.03, 3.23) | 0.82  0.34 |
| R | 1 | 7 | 2 |
| Meropenem | S | 5 | 16 | 10 | 1.00  2.0 (0.34, 11.54)  1.28 (0.27, 5.93) | 0.44  0.75 |
| R | 4 | 10 | 4 |
| Ceftazidime | S | 3 | 14 | 9 | 1.00  3.6 (0.62, 21.03)  2.3 (0.47, 11.34) | 0.15  0.23 |
| R | 6 | 12 | 5 |



**Figure 1:** Agarose gel electrophoresis of the PCR products obtained from optimization of PCR targeting the *sodC* gene.

Lane 1, 1kb+ DNA ladder marker; Lane 2, 7, and 12 are *N. meningitidis* ATCC control strain, Lane 3, 8, and 13; 4, 9, and 14; 5, 10, and 15 are *N. meningitidis*  islotates of serogroup X, Y, and W, respectively amplified by three different versions of *sodC* primers. Lane 6, 11 and 16 are negative controls.



**Figure 2:** Agarose gel electrophoresis of the PCR products obtained from PCR targeting the *sodC* gene and the *ctrA* gene.

Lane 1, 1kb+ DNA ladder marker; Lane -2 *N. meningitidis* ATCC control strain, Lane 3-10 clinical samples amplified by primers targeting the *sodC*, Lane 11, negative control.; and Lane 12–20 clinical samples amplified by primers targeting the *ctrA* gene.