
Antibiotic Susceptibility Patterns of *Neisseria Meningitides* Isolates from Asymptomatic Carriers in Gurage Zone, Southern Ethiopia

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Abstract: *Neisseria meningitides* represents a pathogen of great public health importance in both developed and developing countries. Resistance to some antimicrobial agents used either for therapy of invasive infections or for prophylaxis of case contacts has long been recognized. However, there is no data in relation with the circulating serotypes and antimicrobial resistance patterns of *Neisseria meningitides* in Ethiopia. Therefore; the aim of this study was to assess drug susceptibility patterns of *Neisseria meningitides* from asymptomatic carrier for all age group at Meskan and Mareko Districts, Gurage Zone, in the Southern Nations, Nationalities and Peoples Regional State Ethiopia. A Cross-sectional survey of an age-stratified population in Meskan and Mareko Districts, Gurage Zone, in the Southern Nations, Nationalities and Peoples Regional State in Ethiopia was conducted at AHRI as part of the MenAfrican project. A total of 4110 subjects were screened and from these 187 *Neisseria meningitides* positive isolates was selected for Antimicrobial susceptibility testing (AST). Antimicrobial susceptibility test was done on stored *Neisseria meningitides* isolates. The activities of ten antimicrobial agents used for treatment and prophylaxis of meningococcal disease were investigated. The AST was performed for *Neisseria meningitides* isolates according to the criteria of the CLSI guide line by disk diffusion method. Data were analysed by using SPSS version 20.0 software. From 187 isolates 8(4.28%) were serogroup X, 24(12.83%) were serogroup Y, 1(0.53%) were serogroup W135, and 154(82.35%) were non determinant (ND). Cotrimoxazol resistant were the highest accounting 116(62%), Ciprofloxacin resistant were 112(60%), Cefotaxime resistant were 26(14%), Ceftriaxone resistant were 24(13%), Meropenem resistant were 21(11%), Minocycline resistant were 15(8%), Rifampine resistant were 149(7%), 10(5%) were resistant to Azithromycin, 7(4%) were resistant to Chloramphenicol and 6(3%) were resistant to Levofloxacin and 102(54.5%) isolates were resistance for more than one drug. So, it has been concluded that an antimicrobial susceptibility pattern of *Neisseria meningitides* among asymptomatic carriers is high and continued surveillance of meningococci for antimicrobial resistance is necessary to monitor early detection of changes in susceptibility patterns that might affect recommendations for chemoprophylaxis and treatment.

Keywords: *Neisseria Meningitides*, Serogroups, Sensitivity Pattern, Drug Resista

1. Introduction

Infections by *Neisseria meningitides* are significant causes

of mortality and morbidity in young children and adolescents. The epidemiology of serious meningococcal disease is an area of considerable interest, and many unanswered questions

surround this organism and the types of diseases it causes. Group A and group C meningococci are frequently the cause of major epidemic disease, particularly in underdeveloped countries and among the poorer segments of society, perhaps reflecting certain risk factors associated with transmission, such as crowding and poor sanitation. The organisms may be asymptotically carried in the oropharynx and nasopharynx of a variable percentage of individuals, and the rate of carriage is related to several factors such as age, socioeconomic class, and the presence of actual disease in a community [1].

Neisseria meningitidis is not only a common bacterial commensal of the human upper respiratory tract (nasopharynx) but also an important and devastating human pathogen. Meningococci are gram-negative diplococci, can be encapsulated or unencapsulated, have a genome of about 2.1–2.2 m bases with roughly 2000 genes, and are the worldwide cause of epidemic meningitis and rapidly progressing fatal shock. The persistence of large serogroup A outbreaks in Africa, the emergence in different regions of serogroups Y, X, and W-135 in the past decade, and the persistence of serogroups B and C disease in many industrialized countries [2].

Studies of meningococci isolated from the nasopharynx, which is the normal environment of the meningococcus, are essential to improve knowledge of the epidemiology of meningococcal disease. The results of carriage studies, however, are highly dependent on the swabbing techniques and laboratory methods used. Swabbing of the posterior wall of the oropharynx, followed by immediate cultivation on selective medium, is the recommended procedure to detect asymptomatic meningococcal carriage in an individual. Some real-time PCR methods have been attempted more recently, but their sensitivity is not greater than the microbiological techniques based on culture [3].

Antimicrobial treatment and chemoprophylaxis for patients with meningococcal disease and their close contacts is critical to reduce morbidity and mortality and to prevent secondary cases. Although an extended-spectrum cephalosporin such as ceftriaxone is recommended for empirical treatment of meningitis, some treatment guidelines recommend switching to penicillin G when *Neisseria meningitidis* is confirmed. Ceftriaxone, ciprofloxacin, and rifampin are the currently recommended chemo prophylactic antimicrobials. Azithromycin was recommended as an alternative chemo prophylactic antimicrobial in eastern North Dakota and western Minnesota when ciprofloxacin resistance was first reported in North America (serogroup B) [4].

Early antibiotic treatment of meningococcal disease is crucial for keeping the case fatality rate and risk of sequelae as low as possible. Comprehensive data regarding the antibiotic susceptibility of *Neisseria meningitidis* in many African countries are limited, and no up-to-date extensive study of the antibiotic susceptibility of *Neisseria meningitidis* is at hand [5]. The scientific literature contains a wealth of susceptibility data for clinical meningococci isolates associated with a variety of medical conditions. There is, however, a lack of data that describe the

antimicrobial susceptibilities of *Neisseria meningitidis* strains that colonize the nasopharynx. Knowledge of susceptibility patterns and of trends in the resistance of colonizing strains may be of great value in establishing a policy for empirical antimicrobial treatment of meningococcal disease (MD) and in developing appropriate prophylactic regimens for eradication of the carrier state in persons at high risk of developing serious infection [6]. There are limited data on the antimicrobial susceptibility pattern of *Neisseria meningitidis* in Ethiopia in general and in the study settings in particular. Hence this study gave us a good indication to know AST pattern of *Neisseria meningitidis* in our country and it is a crucial and must know the problem to improve the drug administration.

2. Methods

2.1. Study Area

The study was conducted on isolates collected from Meskan district in Gurage Zone, Southern Nations, Nationalities and Peoples Regional State in Ethiopia. The estimated area of the district is 797 km², of which Butajira covers about 9 km². The area is found 130 km south of Addis Ababa and 50 km west of Ziway in the Rift Valley, latitude 8.2°N and longitude 38.5°E. The district includes arid lowland areas, at altitudes of around 1,500 m above sea level, which have a tropical climate, and cool mountainous areas of up to 3,500m, which have a more temperate climate. The district has an estimated population of 260,000, with a density of around 325 people/km².

2.2. Study Design and Period

A retrospective study on isolates collected for a MenAfrican project at AHRI Cross-sectional survey of an age-stratified population of 4110 subjects conducted during the dry season and during the rainy season was done.

2.3. Source Population

Isolates collected during MenAfrican project in Meskan and Mareko Districts, Gurage Zone, in the Southern Nations, Nationalities and Peoples Regional State in Ethiopia.

2.3.1. Study Population

Neisseria meningitidis isolates collected from MenAfrican project at AHRI.

2.3.2. Inclusion and Exclusion Criteria

Inclusion Criteria: *Isolated samples positive for Neisseria meningitidis.*

Exclusion criteria: *Isolated samples which are auto and poly agglutinate for Serotype.*

2.3.3. Variables of the Study

Dependent Variable: *Antimicrobial Susceptibility pattern of Neisseria meningitidis.*

Independent Variables: *Socio demographic characteristics Serogroups of Neisseria meningitidis*

2.4. Sample Size and Sampling Methods

All stored isolates which were confirmed as *Neisseria meningitides* were included except poly and auto agglutinate isolates by using convenient method.

2.5. Data Collection

2.5.1. Information Collection for Positive *Neisseria Meningitides* Isolate

Data collected from AHRI bacteriology laboratory log book to gather information, we have seen first AHRI identification code, Gram staining, Serogroup, Bio chemical test. (oxidase positive, Gram negativediplococci (OPGND), ONPG negative, GGT positive and Tributyrin negative bacteria) to be sure they were typically *Neisseria meningitides* isolates.

2.5.2. Sociodemographic Data

The socio demographic data collected from AHRI data room we collected necessary information for this study. it was annexed.

2.5.3. Sub Culturing of *Neisseria Meningitides*

We had information from bacteriology log book, Samples were stored at -80°C. and preserved by BHI (brain heart infusion) with beads we took one bead and spread or subculture on blood agar (BA) and incubated in 5% CO₂ at 37°C for 18-24 hours.

2.5.4. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing carried out on isolates of *Neisseria meningitides* by using disc diffusion technique on Mueller-Hinton agar supplemented with 5% sheep blood. then when we get bacterial growth a suspension of the test organism prepared by taking 3-5 colonies from blood agar plate by emulsifying in 1 ml of sterile physiological saline and incubated at 37°C until the turbidity of the suspension become matched with turbidity standard equivalent to 0.5 McFarland. Using a sterile swab, the surface of Mueller-Hinton with 5% sheep blood agar will be completely covered by pressing and rotating the swab against the side of the tube above the level of suspension. After the plate has dried (3-5 minutes), using sterile forceps the discs will be evenly distributed on the inoculated plate and incubated in 5% CO₂ at 37°C for 24 hours according to the guideline recommendations of CLSI-2014. Antibacterial susceptibility test for numbers of antibiotics which including; Ceftriaxone (30µg), Cefotaxime (30µg), Meropen (10µg), Azithromycin (30µg), Minocycline (30µg), Ciprofloxacin (5µg), levofloxacin, Trimethoprim sulfamethoxazole (1.25/ 23.75 µg), Chloramphenicol (30µg), and Rifampin (5µg), by disc diffusion technique (Kirby-Bauer method). The result recorded by measured zone of inhibition growth by Using a ruler, The plates were incubated in aerobic atmosphere at 37°C for 24-48 hours. Diameters of the zone of inhibition around the disc are measuring to the nearest millimeter using a graduated caliper in millimeters, and the isolates classified as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI (CLSI, 2014) [7].

Recommended precautions: Perform all antimicrobial susceptibility testing (AST) of *Neisseria meningitides* in a biological safety cabinet (BSC). Manipulating *Neisseria meningitides* outside a BSC is associated with increased risk for contracting meningococcal disease. Exposure to droplets or aerosols of *Neisseria meningitides* is the most likely risk for laboratory-acquired infection. Rigorous protection from droplets or aerosols is mandated when microbiological procedures (including AST) are performed on *Neisseria meningitides* isolates. If a BSC is unavailable, manipulation of these isolates should be minimized, limited to Gram staining or serogroup identification using saline solution, while wearing a laboratory coat and gloves and working behind a full-face splash shield.

2.6. Data Management

The data obtained kept on a secured, password protected computer and back up data also kept at Addis Ababa University (AAU), College of Health Science (COHS), School of Allied Health Science (SOAHS), Department of Medical Laboratory Sciences (DMLS) and Armaur Hansen Research Institute (AHRI)/ All Africa Leprosy, Tuberculosis and Rehabilitation Training (ALERT). Hard copies of the data collection worksheets was kept securely locked for the duration of the study after which archived to protect client confidentiality.

2.7. Data Analysis

Data analysis and cleaning was done by using SPSS version 20.0 software. Data was double entered to check consistency. Frequency count and percentage used to clean and check the accuracy of data entry. Prevalence figures calculated for the total study population and separately by age groups. Chi-square test used compare results between participants with different age groups and with the previous findings from the literature. P-value less than 0.05 were considered statistically significant.

2.8. Quality Assessment

The performance of culture media were controlled by using quality control strains *S. pneumoniae* ATCC 49619. The organism identified based on colony characteristics and identification tests. The quality of the test was assessed by testing of *S. pneumoniae* ATCC 49619 strains on each new lot/shipment of disks, Quality controls (QC) of antibiotic susceptibility tests were used and performed in accordance with laboratory standards using reference strains: *S. pneumoniae* ATCC 49619 before or concurrently with placing these materials into use for testing bacteria isolates. Any out of range result is immediately investigated and corrective action performed prior to release result.

The quality of our work assessed by first seeing the sterility of culture media was checked by incubating 3-5 % of the batch at 35 – 37°C overnight to see contamination by any organisms. Those media which showed growth were discarded. *S. pneumoniae* ATCC 49619 were used as the quality control strains for each run as recommended by

CLSI-14 guide lines.

2.9. Ethical Consideration

The proposal was approved and ethically cleared by Department of Ethics and Research Committee (DERC) of Addis Ababa University (AAU), College of Health Science (COHS), School of Allied Health Sciences (SOAHS), Department of Medical Laboratory Science (DMLS) and AHRI/ ALERT Ethical Review Committee (AAERC). As this was a study on stored samples, we obtained the AAERC for waiver of consent.

3. Results

3.1. Socio Demographic Characteristics

In the present study, *Neisseria meningitidis* were isolated from all age groups. Among 187 *Neisseria meningitidis* positive isolates, 99(53%) were males and 88 (47%) were females. The highest carriage rate was observed among the age groups of 1-10 (33%) and 11-20 years (33%). The lowest rate was observed among age groups of < 1years old (3%). The highest carriage rate was observed in rural area 128(68%) than urban area 59(32%) (Table 1).

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

Characteristics of the study population (n=187)		N	%
Sex	Male	99	53
	Female	88	47
Residence	Urban	59	32
	Rural	128	68
	<1	6	3
	1-10	61	33
Age	11-20	61	33
	21-30	25	13
	31-40	17	9
	>41	17	9

3.2. Serogroup Distribution of Bacterial Isolates

In this study serotype Y accounted 12.8% (24) and majority of them 82.3 % (154) were non-determinant (Table 2).

Table 2. Serogroup Distribution of *Neisseria meningitidis*.

Serogroup	Freq.	Percent
ND	154	82.35
W-135	1	0.53
x	8	4.28
y	24	12.83
Total	187	100.00

3.3. Antimicrobial Susceptibility Patterns of Bacterial Isolates

The Antimicrobial susceptibility patterns of all positive for *Neisseria meningitidis* isolates is shown in table 3. About 62% (116/187) of *Neisseria meningitidis* isolates were Cotrimoxazole and the least resistance were observed 6(3%) for Levofloxacin. Moreover, *Neisseria meningitidis* isolates showed resistance to any of one drug was 49(26.2%), 102

(54.5%) isolates were multidrug resistance (resistance to more than one antibiotic) and 36(19.3%) isolates were susceptible.

Table 3. Drug susceptibility profile of *Neisseria meningitidis* (No=187).

Antimicrobials		N	%
Azithromycine/AZM	I	30	16
	R	10	5
	S	147	79
Cefotaxime/CTX	I	12	6
	R	26	14
	S	149	80
Ceftriaxone/CRO	I	11	6
	R	24	13
	S	15	8
Ciprofloxacin/CPR/CIP	I	11	6
	R	112	60
	S	64	34
Chloramphenicol	I	53	28
	R	7	4
	S	127	68
Meropenem/M EM	I	7	4
	R	21	11
	S	159	85
Levofloxacin	I	3	2
	R	6	3
	S	178	95
Rifampine/RD	I	42	22
	R	14	7
	S	131	70
Trimethoprim sulfamethoxazole/SXT	I	8	4
	R	116	62
	S	63	34
Minocycline	I	15	8
	R	15	8
	S	157	83

The AST patterns of *Neisseria meningitidis* was assessed with gender and there is no significant difference between AST pattern and gender of the study participants. (Table 4)

Table 4. Association between Gender and Antibiotic Susceptibility Patterns.

Azithromycine			
Sensitive	95(96%)	82 (93%)	0.399
Resistant	4(4%)	6 (7%)	
Cefotaxim			
Sensitive	86(87%)	75 (85%)	0.746
Resistant	13(13%)	13 (15%)	
Ceftriaxone			
Sensitive	86(87%)	77 (87.5%)	0.897
Resistant	13(13%)	11 (12.5%)	
Ciprofloxacin			
Sensitive	39(39%)	36 (41%)	0.833
Resistant	60(61%)	52 (59%)	
Chloramphenicol			
Sensitive	95(96%)	85 (97%)	0.820
Resistant	4(4%)	3 (3%)	
Meropenem			
Sensitive	88(89%)	78 (89%)	0.956
Resistant	11(11%)	10 (11%)	
Levofloxacin			
Sensitive	97 (98%)	84 (95%)	0.328
Resistant	2 (2%)	4 (5%)	

Rifampine			
Sensitive	94 (95%)	79 (90%)	0.179
Resistant	5 (5%)	9 (10%)	
Trimethoprim sulfamethoxazole			
Sensitive	38 (38%)	33 (37.5%)	0.901
Resistant	61 (62%)	55 (62.5%)	
Minocycline			
Sensitive	92 (93%)	80 (91%)	0.612
Resistant	7 (7%)	8 (9%)	

Similarly, the antibiotic resistance for specific drugs are different for all age groups but highest percentile of resistance seen at age group 11≤20 years for Trimethoprim sulfamethoxazole and the lowest resistance was seen for age group <1years old however, there is no significant difference between age group and AST patterns (table 5).

Table 5. Association between age and Antibiotic Susceptibility Patterns.

Drugs	Age group in year												p-value
	<1		1 <10		11 < 20		21 <30		31 < 40		>40		
	R	S	R	S	R	S	R	S	R	S	R	S	
Azithromycine	0	6	2	59	5	56	0	25	2	15	1	16	0.462
Cefotaxim	2	4	11	50	7	54	4	21	1	16	1	16	0.418
Ceftriaxone	1	5	12	49	6	55	3	22	0	17	2	15	0.341
Ciprofloxacin	3	3	38	23	41	20	14	11	5	12	11	6	0.124
Chloramphenicol	0	6	1	60	4	57	0	25	1	16	1	16	0.589
Meropenem	3	3	5	56	8	53	3	22	1	16	1	16	0.055
Levofloxacin	0	6	3	58	1	60	1	24	0	17	1	16	0.812
Rifampine	0	6	7	54	5	56	1	24	0	17	1	16	0.577
Trimethoprim sulfamethoxazole	4	2	37	24	41	20	15	10	8	9	11	6	0.775
Minocycline	0	6	5	56	5	56	1	24	2	15	2	15	0.886

3.4. Multiple Drug Resistance Patterns

The findings showed 55(29.4%) of isolates were resistance to two drugs, 9(4.8%) were resistance to four drugs. From the total number of isolates, serogroup ND 13/154 isolate were resistance for more than five drugs (Table 6).

Table 6. Multi-drug resistance pattern for *Neisseria meningitides* isolates.

Serogroup	Anti-microbial sensitivity pattern							Total
	R0	R1	R2	R3	R4	≥R5		
ND	31	38	45	19	8	13	154	
X	2	2	2	1	-	1	8	
Y	3	9	7	3	1	1	24	
W-135	-	-	1	-	-	-	1	
Total	36	49	55	23	9	15	187	
Percentile	19.3%	26.2%	29.4%	12.3%	4.8%	8%	100%	

R0: no resistance, R1: resistance to one drug, R2: resistance to two drug, R3: resistance to three drugs, R4: resistance to four drugs, ≥R5: resistance to five and above drugs.

4. Discussion

Neisseria meningitides represents a pathogen of great public health importance in both developed and developing countries. Resistance to some antimicrobial agents used either for therapy of invasive infections or for prophylaxis of case contacts has long been recognized [8].

Increasing antimicrobial resistance among bacterial pathogens is a serious public health threat worldwide and today resistance can be found in almost every bacterial species for which antibiotic therapy exists [9].

In the present study isolates were used from all age groups and 53% were males and 47% were females and the residences of 68% of them were from rural.

The prevalence of *Neisseria meningitides* in our study was 320(7.8%) from the total population we used it is comparable

to the study conducted in Ethiopia by Bärnes GK, et al. (10) in Arba Minch, southern Ethiopia 6.6%, in Burkina Faso 7.86% (11), in Turkey by Gazi H. et al. 6.2% [1] and study conducted in Greece (7.2%) [12]. In contrary higher prevalence of carriage rate reported in India (10.4%) [13] And lower nasopharyngeal carriage of *Neisseria meningitides* was reported in healthy Dutch children 46 (1.5%) [12] And in Greece children (4.0%) [14]. our results showed higher nasopharyngeal carriage rate of *Neisseria meningitides* among older children less than twenty years old. The high prevalence of *Neisseria meningitides* could be due to the fact that many children may be immune debilitated due to the diseases, which were the reason for children to visit the hospital. This may, therefore, suggest that large numbers of meningococcal carriers are at high risk of developing invasive meningococcal diseases and the family members, their peers with whom they interact in the community are at risk of acquiring the pathogens.

The finding showed only 36/187(19.3%) isolates were not having resistance for drugs what we used, the others 151(80.7%) had drug resistance which indicates more than eighty percent of our isolates were resistance for standard antibiotic for these bacteria. Moreover, *Neisseria meningitides* isolates showed resistance to one drug was 49(26.2%), multidrug resistance (resistance to more than one antibiotic) was 102 (54.5%).

Neisseria meningitides isolates resistant were 116(62%) to Cotrimoxazol, 112(60%) to Ciprofloxacin, 26(14%) to Cefotaxime, 24(13%) to Ceftriaxone, 21(11%) to Meropenem, 15(8%) to Minocycline, 14(7%) to Rifampine, 10(5%) to Azithromycine, 7(4%) to Chloramphenicol and 6(3%) to Levofloxacin.

Contrary to the present study, a study done by Assefa et al.(15) all isolated *Neisseria meningitides* were susceptible to chloramphenicol, erythromycin and tetracycline but similar

to our findings, even if they used small number of isolate, the highest resistance rates of *Neisseria meningitides* against cotrimoxazole were 14 (100%), ceftriaxone were 7 (50.0%) and ciprofloxacin were 3 (21.4%).

Reduced susceptibility to ciprofloxacin and resistance to rifampicin have also been reported from many countries. Resistance to ceftriaxone is claimed to have been identified and was reported from India [9].

Different to our findings study done by Rohani et al. showed that all strains subjected to antibiotic sensitivity testing were susceptible to chloramphenicol, rifampin, levofloxacin, cefotaxime and penicillin. The rate of resistance to cotrimoxazole was 84%. Resistance to trimethoprim-sulphamethoxazole was high among these isolates and the rate was almost similar to our reports. The widespread resistance to this antibiotic is possibly due to the early introduction of sulphonamides [16].

Contrary to our study in Greek children in 2004 showed all isolates were sensitive to ceftriaxone, rifampicin and chloramphenicol. Only one (4.5%) isolate was resistant to cotrimoxazole, while five (22.7%) showed intermediate resistance to penicillin [14].

Another study conducted on Cankaya municipality schools of Ankara province in 2005 the antibiotic susceptibilities of *Neisseria meningitides* isolates were determined for penicillin, sulfadiazine, rifampicin, and azithromycin. The resistance against sulfadiazine was 54.4%, while it was 26.9% against azithromycin. No rifampicin-resistant strains were detected. But ours were less resistance for azithromycin (5%) and 14(7%) of them were resistance for rifampicin.

Another study from Punjab (Ludhiana) has studied 84 Isolates obtained; all the isolates were sensitive to penicillin, ampicillin, rifampicin and ceftriaxone. As regards to ciprofloxacin, about two third of the isolates tested were found to be 'nonsusceptible'. All the isolates were found resistant to cotrimoxazole [17].

Furthermore, it was observed that pathogenic strains were genetically related to isolates from carriers. Since asymptomatic carriers are presumably the major source of transmission of pathogenic strains, eradication of the carriage state may result in a significant reduction in invasive meningococcal disease [14].

Therefore, this result noted that *Neisseria meningitides* is developing a resistance to antibiotics, appropriate for management of meningococcal meningitis for epidemic response. However, the overall finding of our result showed relatively other than the two drugs (SXT and CIP) the others are effective antibiotics in the included community.

5. Conclusion

Almost 122 (65%) isolates of *Neisseria meningitides* showed higher resistance rate for ciprofloxacin and Trimethoprim sulfamethoxazole. There was an indication of higher cotrimoxazole and ciprofloxacin resistances and also to other drugs.

It has been noted that carriage studies are important to

improve the understanding of drug susceptibility patterns of *Neisseria meningitides* in the study settings.

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