

## Isolation and antibiotic susceptibility profile of *Neisseria gonorrhoeae* isolated from urine samples in Zaria, northern Nigeria.

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### Abstract

One hundred and fifty urine samples (early morning midstream urine void) from students, commercial sex workers, commercial drivers and illiterates men and women in Zaria, Nigeria were collected for this study. These urine samples were cultured and the *Neisseria gonorrhoeae* isolates identified using conventional microbiological media and methods.

Of these samples collected, only 38.00% ( $^{(7/150)}$ ) were found to contain *Neisseria gonorrhoeae* usually implicated in sexually transmitted disease. This result showed that students from Kaduna State Polytechnic Zaria have high rate of *N.gonorrhoeae* isolates (80.00%) than students from Faculty of Education, A.B.U, Zaria (20.00%). This observed variation in *N.gonorrhoeae* isolation rate may probably be related to the impact of the level of health education acquired by the participants in this investigation.

The antibiotic susceptibility profile for the isolated *N.gonorrhoeae* showed emergence of resistant isolates to Penicillin, Quinolone, Tetracycline, Ampicillin, Gentamicin, and Spectinomycin.

Thus, this result has shown the need for prudent use of antibiotics and it's evaluation before prescription is made for treating sexually transmitted infection.

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### Introduction

Clinical infection of the urinary tract is said to exist when a significant number of microorganisms usually approximately  $10^5$  cells / ml of urine are detected from properly collected midstream "clean catch" urine or from a catheter specimen (1). The infections of the genital tract are among the most common infectious diseases in adult human, because of their sexual activities. It has been reported in southern Nigeria that, the main factors predisposing bacteria infection in genital tract of men, women, commercial sex workers, and schoolboys and girls has been attributed to sexual intercourse (2). Thus, this study was carried out to investigate the prevalence and antibiotic susceptibility of *N. gonorrhoeae* isolates from the urine samples of boys and girls in higher institutions, commercial sex workers, commercial drivers, and illiterates in Zaria, Northern Nigeria.

### Materials and methods

Fifteen men and women each from Kaduna State Polytechnic Zaria, Faculty of Pharmaceutical Sciences, Faculty of Education both of Ahmadu Bello University Zaria, illiterate men and women from Samaru Village, Zaria, Commercial sex workers and commercial drivers urine samples were used in this study.

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Two sterilized capped bottles of 50ml volume each was given to each volunteer. The first clean catch of urine samples collected by each volunteer in sterile clean capped bottles was stored in sterile plastic containers at ambient temperature of  $25 \pm 20^\circ\text{C}$ . Each urine sample on arrival at the laboratory within 2hours of collection was immediately centrifuged at 5000 revolution per minute aseptically. The supernatant was discarded and the sediment re-suspended in one milliliter of sterile normal saline. The re-suspended sediment from each urine sample was aseptically inoculated in triplicates into chocolate agar plates (2). These were incubated in an anaerobic condition at  $37^\circ\text{C}$  for 18hours (2). The characteristics of *N.gonorrhoeae* isolates were observed on the selective medium, the isolates were subjected to microscopical and biochemical tests for proper identification (3).

### Antibiotic Susceptibility

Antibiotic susceptibility test of fifty-seven *N.gonorrhoeae* isolates against ten commonly prescribed antibiotics [Spectinomycin (SPT  $100\mu\text{g}$ ), Gentamicin (GM  $10\mu\text{g}$ ), Erythromycin (E1  $15\mu\text{g}$ ), Amoxicillin (AMC  $30\mu\text{g}$ ), Ampicillin (AM  $10\mu\text{g}$ ), Amikacin (AN  $30\mu\text{g}$ ), Penicillin (P  $10\mu\text{g}$ ), Streptomycin (S1  $10\mu\text{g}$ ), Tetracycline (TE  $30\mu\text{g}$ ) and Ciprofloxacin (CIP  $5\mu\text{g}$ )], was determined using the official microbiological protocol of agar diffusion method (3,4).

Standardized overnight culture of *N. gonorrhoeae* isolate  $10^6$  cells / ml was used to seed melted Mueller Hinton Agar (MHA) at  $45^\circ\text{c}$  and poured into sterilized plates in triplicates aseptically. These were allowed to solidify. Antibiotic discs were then aseptically placed at reasonable equidistant on the seeded MHA plates and allowed to stand for one hour. These were then incubated at  $37^\circ\text{c}$  for 18hours in an anaerobic condition.

The diameter of zone of inhibition produced by each antibiotic disc was measured using engineer calipers and the result interpreted (5) as sensitive (S) or resistant (R) in the test antibiotic agent used, depending on the length of zone of inhibition produced compared to the reported standard length. (5) The multiple antibiotic resistance (MAR) index for *N.gonorrhoeae* isolate was calculated as recommended by Krumpferman (6).

## Results

A total of one hundred and fifty urine samples of males and females were screened for the presence of *N.gonorrhoeae* in this study.

Fifty-seven urine samples showed the presence of *N.gonorrhoeae* with twenty-three isolates from men and thirty-four from women with x implicated in sexual transmitted disease (Table 1.0).

**Table 1.0: Summary of *N. gonorrhoeae* isolation and antibiotic sensitivity from urine samples of men and women in Zaria, Northern Nigeria**

Gender Screened	N. Gonorrhoeae Isolates Sensitivity profile	
	(S)	(R)
Male (75)	0 (0.00%)	23/23 (100%)
Female (75)	4/34 (11.76%)	30/34 (88.24%)
Total (150)	4/57 (7.02%)	53/57 (92.98%)

**Key:** S = Sensitivity MAR = 0.20 R = Resistant MAR = 0.20

All the *N.gonorrhoeae* isolates from the urine samples of men (75) were resistant<sup>23/23</sup> (100%) while isolates from women (75) also displayed multiple antibiotic resistance<sup>30/34</sup> ((88.24%). The antibiotic susceptibility profiles of *N.gonorrhoeae* isolates indicate that Ciprofloxacin and Gentamicin produced the greatest in-vitro activities. Spectinomycin, a reserved drug in the therapy of gonococcal infection was considerably ineffective [92.98% resistant, Table 2.0]. Other antibiotics such as Penicillin, Amoxycillin, Ampicillin and Streptomycin did not show any appreciable effectiveness when compared to Gentamicin and Ciprofloxacin.

Table 2. 0: Susceptibility profiles of *N. gonorrhoeae* isolates from urine samples to ten antibiotics

Antibiotics	Susceptibility Profiles	
	S	R
Penicillin	0	57 (100%)
Amoxycillin	0	57 (100%)
Erythromycin	0	57 (100%)
Tetracycline	0	57 (100%)
Amikacin	0	57 (100%)
Streptomycin	2 (3.51%)	55 (96.49%)
Ampicillin	2 (3.51%)	55 (96.49%)
Spectinomycin	4 (7.02%)	53 (92.98%)
Ciprofloxacin	36 (63.16%)	21 (36.84%)
Gentamicin	51 (89.47%)	6 (10.53%)

**Key:**

S = Isolates sensitive to test antibiotics such as Gentamicin and Ciprofloxacin according to NCCL reports 1993

R = Isolates resistant to test antibiotics such as Penicillin, Amoxycillin, Erythromycin, Tetracycline, Amikacin e.t.c. according to NCCL reports 1993.

The MAR indices of the investigated *N.gonorrhoeae* showed that the majority of the isolates were resistant to more than three antibiotics (Table 3.0)

Table 3.0: Multiple antibiotic resistance indices of the *n. gonorrhoeae* isolates From fifty-seven urine samples

MAR Index	Frequency of MAR Index
0.00	0.0 (0.00%)
0.10	17.00 (29.82%)
0.20	36.00 (63.16%)
0.30	4.00 (7.02%)
0.40	0.00 (0.00%)
0.50	0.00 (0.00%)

It is also important to note that the order of *N.gonorrhoeae* isolates from urine sample from different volunteer groups were students from Kaduna State Polytechnic, Zaria 80.00% (<sup>24/30</sup>), female commercial sex workers and male commercial drivers 66.67% (<sup>20/30</sup>), students from Faculty of Education A.B.U, Zaria 20.00% (<sup>16/30</sup>), uneducated men and women in Zaria 13.33 (<sup>4/30</sup>) and students from Faculty of Pharmaceutical Sciences, A.B.U, Zaria 10.00% (<sup>3/30</sup>) respectively.

Generally all the *N. gonorrhoeae* isolates displayed high level of multiple antibiotics resistance. (Table 4.0)

**TABLE 4.0: Summary of *N. gonorrhoeae* isolates and susceptibility profile from Different groups of volunteers urine samples.**

Volunteers Groups (Male and Female= 30)	Isolation Rate Per Center (%)	Susceptibility Profile (%)	
		Sensitive Isolates	Resistant Isolates
Kaduna State Polytechnic Zaria	24 (80.00)	0.00	100.00
Faculty of Education	6 (20.00)	0.00	100.00
Faculty of Pharmaceutical Sciences	3 (10.00)	0.00	100.00
Female Commercial Sex Workers and Male Commercial Drivers	20 (66.67)	20.00	80.00
Uneducated Men and Women in Zaria	4 (13.33)	0.00	100.00

## Discussion.

The result of *N. gonorrhoeae* isolation from urine samples of one hundred and fifty volunteers in Zaria, Northern Nigeria indicated a high level of *N. gonorrhoeae* isolation rate of 38.0%. This high prevalence of *N. Gonorrhoeae* in the genital tract have been implicated in infertility in women in Ile-Ife, Western Nigeria.(7) This observation of high rate of *N. gonorrhoeae* isolation appears to suggest a serious gonococcal problem that require prompt action.

Generally, *N. gonorrhoeae* isolation rate was more in the women examined (59.65%) than the men (40.35%). This difference may not be unconnected to the fact that early manifestation of this sexually transmitted disease is more pronounced in men with accompany pain than in women, hence the search for medical advise to reduce the medical problem (2).

The order of *N. Gonorrhoeae* isolation from the urine samples of the volunteers in the different centres investigated were Kaduna state Polytechnic Zaria students 80.00% ( $\frac{24}{30}$ ) Commercial sex workers and commercial drivers 66.67% ( $\frac{20}{30}$ ), Faculty of Education Students, A.B.U. Zaria 20.00% ( $\frac{6}{30}$ ), Uneducated Men and Women from Samaru, Zaria 13.33% ( $\frac{4}{30}$ ) and faculty of Pharmaceutical Sciences Students, A.B.U. Zaria 10.00% ( $\frac{3}{30}$ ). This result has showed that the students from Kaduna State Polytechnics, Zaria and commercial sex workers and Drivers volunteers were highly infected with *N. Gonorrhoeae* This finding may probably point to the level of promiscuity among these groups of volunteers as a major route of infection. The comparable *N. gonorrhoeae* isolation rate from Kaduna State Polytechnic Students, Zaria and commercial sex workers is a dangerous medical signal, hence the need of Health Education in schools.

This study has also shown that the cultural habits of using shared water plastic containers to wash the genital orifice by the uneducated villagers in Samaru, Zaria have not drastically influence the level of *N. gonorrhoeae* infection from their urine samples as otherwise conjectured before this report (8). The multiple antibiotic resistance (MAR) indices showed that a very large proportion of the *N. gonorrhoeae* isolates have been exposed to several antibiotics. MAR indexing is used as a tool to denote the spread of resistance among bacteria population. It has been reported that when MAR index is greater than 0.20, it indicates that the strain of bacteria isolate originates in an environment where several antibiotics are in use (10). The *N. Gonorrhoeae* isolates investigated were all observed to be resistant to antibiotic such as Penicillin (100%), Erythromycin (100%), Tetracycline (100%) Spectinomycin (93.3%), Ciprofloxacin (36.6%), and Gentamicin (10%). This observation of MAR appears to be similar to Van-Dyck and co-workers report (9) in Kugali, Kinshasha and Abidjan for *N. gonorrhoeae*. This highly endemic condition of bacteria resistance appear to suggest a continuous monitoring of temporal changes in antibiotic resistance in order to ensure

adequate treatment of gonococcal infection and with a resultant reduction in its transmission. The high multiple antibiotic resistance indices of *N. gonorrhoeae* as revealed in this study may be due to prevailing poverty, drug misuse and abuse as reported by Ibrahim and co-worker (10). This high MAR indices may probably explain the frequent failure rate in the successful management of gonococcal infection (2). The sale and distribution of antibiotics in Nigeria is yet to attract appropriate required regulation and enforcement from concerned government agencies. This is because antibiotics are readily available from several illegal outlets such as patent medicine stores, street drug vendors and open markets. Furthermore, a large proportion of the populace hardly patronizes qualified medical practitioners, because of unaffordable medical bills. They therefore, resort to self-medication, patronize medical and paramedical quacks with its attendant risks and implications. These practices are more likely to promote prevalence of multiple antibiotic resistances.

## Conclusion

There is a high level of antibiotic resistance among the bacterial isolates from urine samples of different centres in Zaria, Nigeria. The emergence of *N. gonorrhoeae* resistance to Penicillin, Ampicillin, Tetracycline, Spectinomycin, and Ciprofloxacin calls for a policy of routine screening of antibiotics effectiveness, before prescription to reduce the spread of resistant strains.

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