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Identification and Antimicrobial Susceptibility Patterns of *Neisseria gonorrhoeae*, *Ureaplasma* spp. and *Mycoplasma* spp. Isolated from Tribal Women

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Abstract

Sexually transmitted infections (STIs) are a major public health problem worldwide with significant social and economic implications. Effective control and prevention strategies necessitate a thorough understanding of the prevalence, isolation, and identification of STI pathogens. The present study aims to provide a comprehensive analysis of the isolation, identification, prevalence, and antimicrobial susceptibility pattern of STI pathogens based on culture method analysis. Endocervical /vaginal swab samples from female patients symptomatic for STI were cultured on different selective and differential media and pathogens were identified by colony morphology and biochemical tests. Antimicrobial Susceptibility Test (AST) of isolated and identified culture pathogen was performed by using Kirby-Bauer disc diffusion method. Among 209 endocervical/vaginal swab samples from symptomatic patients, 126 (60.28%) tested positive and 83 (39.71%) negative. *Ureaplasma* spp. (n = 100) was the most prevalent isolate, constituting 79.36% of culture-positive samples, followed by *N. gonorrhoea* (n = 99) at 78.57%, and *Mycoplasma* spp. (n = 41) at 32.54% individually and in combination. AST analysis revealed erythromycin (74%), ofloxacin (69%), and roxithromycin (64%) as the most resistant antibiotics for *Ureaplasma* spp. *N. gonorrhoea* showed the highest resistance to cefixime (78.79%), followed by ofloxacin (75.76%) and erythromycin (69.7%). Azithromycin and erythromycin exhibited 100% resistance against *Mycoplasma* spp. The study provides information on the prevalent bacterial pathogens involved in STIs among women in Anuppur and Shahdol districts, Madhya Pradesh. Understanding the diversity, distribution patterns and antibiotic sensitivity of these pathogens is crucial for developing targeted interventions and effective prevention strategies in such resource-limited areas.

Keywords: Sexually Transmitted Infections, *Neisseria gonorrhoeae*, *Ureaplasma* spp., and *Mycoplasma* spp., Antimicrobial Susceptibility Test, Multidrug Resistance

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INTRODUCTION

Sexually Transmitted Infections (STIs) are clinical syndromes caused by micro-organisms that can be acquired and spread through sexual activity.[1] It has a tremendous impact on national health. Failure to recognize and treat STIs early can lead to major complications and long-term consequences. They are responsible for a substantial proportion of maternal health issues, ectopic pregnancy, termination of pregnancies, fertility problems, infant mortality, and the birth of underweight babies.[2] The World Health Organization reports that there are over 1 million daily cases of STIs worldwide, resulting in an estimated 374 million new infections annually.[3]

Neisseria gonorrhoeae (gonococcus) is the second most prevalent causative agent known to cause STI with a significant global public health impact.[4] Non-gonococci STI pathogens like genital *Mycoplasma* spp. (*Mycoplasma hominis* & *Mycoplasma genitalium*) and *Ureaplasma* spp., which include *Ureaplasma parvum* and *Ureaplasma urealyticum*. These facultative anaerobic microorganisms in the lower urogenital tract are linked to urogenital issues in women, such as cervicitis, cystitis, bacterial vaginosis, pelvic inflammatory disease, chorioamnionitis, postpartum fever, infertility, prematurity, intrauterine growth retardation, and systemic neonatal infections.[5]

In India, it is estimated that approximately 6% of adults are affected by STIs.[6,7] There are differences in STI rates among ethnic and racial groups that are important to consider. The epidemiology of STIs within rural communities seldom has been studied.[8] Limited reporting and diagnostic tools in rural and tribal areas lead to underestimation of STI cases. Despite global advancements in diagnosis and treatment, isolated tribal communities, constituting around 110 million individuals in India, face significant socio-economic and health challenges.[9]

Tribal society adheres to traditional customs, mandating members to follow them. Marriage is generally permitted in Indian society to involve only one man and one woman at a time (monogamy). However, many tribal societies have allowed a man or woman to marry multiple women or men, which is known as polygyny and polyandry, respectively. Many researchers have found that tribal societies have a system of endogamy, multiple sex partners, and unprotected sex.[10,11] As a result, the circumstances are favourable for the transmission of STIs among tribals.

Antibiotics are potent medications used in the treatment of bacterial infections. The inappropriate use of these drugs has led to the proliferation of antibiotic resistance across a wide spectrum of bacteria.[12] STI pathogens are becoming increasingly resistant to antimicrobial agents, a global issue. New medications replace antibiotics that have lost their potency, but new strains emerge with new resistance determinants; this problem affects all antibiotic classes.[13]

As there is no data available on prevalence and antibiotic resistance patterns of causative organisms of STIs among tribal women in the Anuppur and Shahdol districts, Madhya Pradesh, the present study was carried out to address this issue.

METHODOLOGY

Study area

The study was conducted among women in the Anuppur and Shahdol districts of Madhya Pradesh.

Recruitment of subjects

Target Patients were females (age ≤55) visiting the OPD or admitted in the wards of Obstetrics & Gynaecology of the District Hospital, Annupur and Birsamunda Government Medical College, Shahdol, from May 2022 to September 2023, with a known history of reproductive tract infections were enrolled in the study. 209 endocervical/vaginal swab samples were collected and subjected to laboratory analysis. Patients experiencing symptoms included cloudy/bloody discharge, burning micturition, yellow/green discharge, painful intercourse, strong vaginal odour, vaginal itching/irritation, weight loss, lower abdominal pain, and abnormal menstruation was major focus for the collection of samples. A bilingual (Hindi and English) questionnaire/ consent form was filled by patients or staff with patient consent before specimen collection.

Table 1. Interpretation of zones of inhibition of antibiotics by Kirby-Baurer method

Antibiotics	Abbreviation	Class of antibiotics	Disc concen.	Diameter of growth inhibition zone (mm)		
				Sensitive	Intermediate	Resistant
Cefixime	CFM	β-Lactam	15 µg	20 or more	11-19	Below 11
Ceftriaxone	CTR	β-Lactam	30 µg	20 or more	14-19	Below 14
Ciprofloxacin	CIP	Fluoroquinolone	5 µg	25 or more	15-24	Below 15
Levofloxacin	LE	Fluoroquinolone	5 µg	20 or more	12-19	Below 12
Ofloxacin	OF	Fluoroquinolone	5 µg	20 or more	13-19	Below 13
Norfloxacin	NX	Fluoroquinolone	10 µg	18 or more	13-17	Below 12
Tetracycline	TE	Tetracycline	30 µg	22 or more	15-20	Below 15
Doxycycline	DO	Tetracycline	30 µg	26 or more	16-25	Below 16
Azithromycin	AZM	Macrolides	15 µg	19 or more	13-18	Below 13
Erythromycin	E	Macrolides	15 µg	21 or more	12-20	Below 12
Roxithromycin	RO	Macrolides	30 µg	18 or more	13-17	Below 13

Sample collection & transportation

Two endocervical/vaginal swabs were collected from each patient, i.e. sterile cotton swab (PW1280) and HiCulture™ Transport Swabs w/ Stuart Transport Medium (MS306S). To protect the viability of pathogens for isolation, the specimens were put onto a culture medium as soon as possible after collection.

Bacterial culture

The necessary differential and selective media along with supplements were purchased from HiMedia and prepared aseptically as per the instructions given by HiMedia. 0.01 ml of a collected sample is aseptically streaked over various selective and differential medium Thayer Martin agar base (M413), A-7 (Shepard's Differential Agar Base: M1739), Chocolate agar base (M1548), and Urogenital mycoplasma broth base (M1374). The plates were incubated aerobically at 37°C for 24 hours.

Quantitative analysis

The quantitative analysis of pathogens in the swab sample was determined by measuring CFU/ml (colony-forming units per milliliter) on a culture plate using the plate count method, specifically the spread plate technique. Swab samples displaying bacterial growth equal to or exceeding 10^5 CFU/ml were classified as culture-positive and subjected to subsequent identification and confirmatory analysis. Samples that showed either no growth or growth less than or equal to 10^5 CFU/ml were considered as culture-negative.

Isolation, identification, and confirmatory analysis of pathogens

The pathogens were tentatively identified based on the pathogen's growth on various culture media, colour production, and colony morphology. The method for the isolation and identification of *N. gonorrhoeae* infections was based on the laboratory analysis of *N. gonorrhoeae.*[14] For *N. gonorrhoeae*, a rapid biochemical identification test kit (KB008 HiNesseria™ identification kit, HiMedia) with seven conventional biochemical tests and five carbohydrate utilization tests was used. *Ureaplasma* spp. were isolated on A-7 or Shepard's Differential Agar,[15] while a specialized medium, Urogenital Mycoplasma Broth, was used for the isolation and identification of genital *Mycoplasma* spp. The culture response, observed after incubation at 37°C for 48 hours to 8 weeks, indicated *Mycoplasma* spp. growth through a pH shift, identified by a color change in the phenol red indicator. *Mycoplasma* spp. isolation through culture methods may take 2 to 5 days for *M. hominis* and up to 8 weeks *for M. genitalium.*[16]

After the identification, the pathogens were sub-cultured on species-specific media to get pure culture. Antimicrobial Susceptibility Test (AST) of isolated and identified pathogen was performed.

Antimicrobial Susceptibility Test (AST)

The assessment of antimicrobial susceptibility to the isolated and identified bacteria against the commonly prescribed antibiotics, as indicated in Table 1, was evaluated using the disc

diffusion technique, specifically the Kirby-Bauer (KB) method. Sterile Mueller-Hinton agar (MHA) plates were prepared and AST was performed by the method described by Sharma et al.[17]

Statistical analysis

Chi-square test, Pearson and Fisher's exact test was used to analyse the age-wise prevalence and associated symptoms of pathogens. An intra-comparison of antimicrobial susceptibility was statistically analysed with one-way ANOVA using Tukey's HSD post-hoc test. The level of significance was expressed as the p-value ($p < 0.001$, $p < 0.005$, $p < 0.01$ and $p < 0.05$). All statistical analyses were performed using IBM SPSS Statistics 20 (Version 20.0, Armonk, NY: IBM Corporation).

RESULTS

Quantitative analysis of STI pathogens

Among the 209 endocervical/vaginal swab samples collected from symptomatic patients, 126 (60.28%) showed bacteriuria $\leq 10^5$cfu/ml, while 83 (39.71%) had no growth or growth $\leq 10^5$cfu/ml. Polymicrobial culture growth was significant, revealing various pathogen combinations. Figure 1 illustrates prevalent combinations, aiding understanding of their distribution in culture-positive samples (n=126). *N. gonorrhoea* + *Ureaplasma* spp. was most common (49.21%), followed by *N. gonorrhoea*, *Ureaplasma* spp., and *Mycoplasma* spp. (19.05%). *Ureaplasma* spp. alone (9.52%), *N. gonorrhoea* alone (8.73%), and *Mycoplasma* spp. alone (10.32%) were detected. *Ureaplasma* spp. (79.36%) was the most prevalent isolate, followed by *N. gonorrhoea* (78.57%) and *Mycoplasma* spp. (32.54%) based on colony morphology and biochemical diagnosis in both single and combined growth.

Pathogen identification and confirmatory analysis

Figure 2 provides an overview of the identification and confirmation of *N. gonorrhoea*, *Ureaplasma* spp., and *Mycoplasma* spp. It includes information on the colony morphology/colour changes of pathogens and the specific biochemical tests used for their identification. *N. gonorrhoea* on chocolate media exhibits small, smooth, translucent, raised convex colonies measuring 0.5-1.0 mm. On modified Thayer Martin agar, colonies are small, greyish-white to colourless, mucoid, and well-defined. After 48 hours, colonies grow to 3mm with a less smooth texture. Biochemically, *N. gonorrhoea* is non-urease-producing, ONPG and VP tests are negative, but oxidase and catalase tests are positive. Nitrate reduction is negative, indicating no nitrate reduction, while the glucose test is positive. Lactose, maltose, sucrose, fructose, and mannose fermentation tests are negative.

Ureaplasma spp. is confirmed by identifying typical colonies on A-7 agar, a

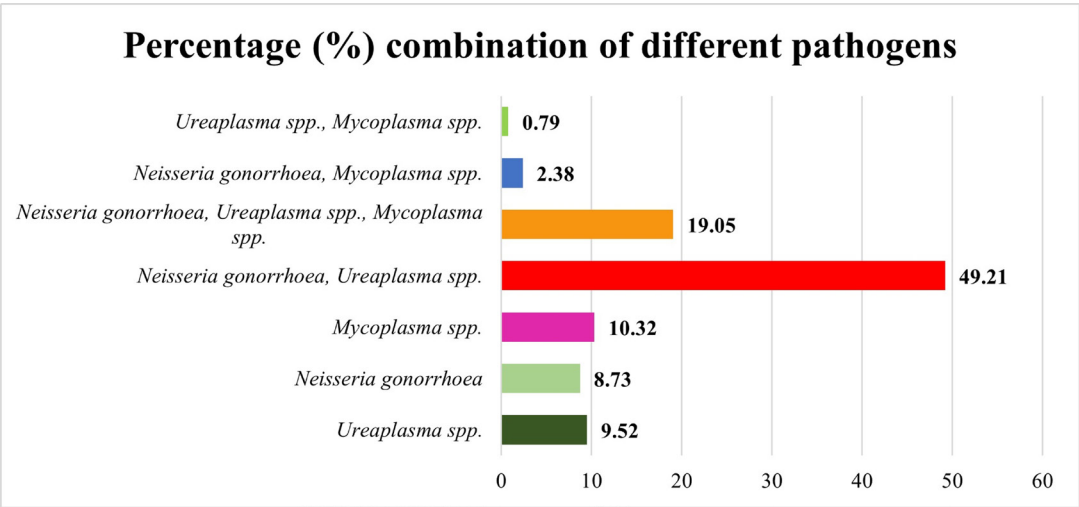


Figure 1. Combination of different pathogens isolated from culture positive (n = 126) samples

Table 2. Age-specific distribution and prevalence of STI pathogens

Samples	Age Groups (Years)						
	≤20	21-27	28-34	35-41	42-48	49-55	p-value
Total Sample Collected (n=209)	5 (2.39%)	34 (16.27%)	59 (28.23%)	69 (33.01%)	28 (13.40%)	14 (6.70%)	-
Culture Positive (n=126)	4 (80%)	21 (61.76%)	33 (55.93%)	45 (65.22%)	16 (57.14%)	7 (50%)	-
Culture Negative (n=83)	1 (20%)	13 (38.23%)	26 (44.07%)	24 (34.78%)	12 (42.86%)	7 (50%)	-
Ureaplasma spp. (n=100)	4(100%)	17 (80.95%)	29 (87.88%)	34 (75.56%)	11 (68.75%)	5 (71.43%)	0.498
Neisseria gonorrhoea (n=99)	4 (100%)	16 (76.19%)	26 (78.79%)	34 (75.76%)	13 (81.25%)	6 (85.71%)	0.740
Mycoplasma spp. (n=41)	1 (25%)	6 (28.57%)	11 (33.33%)	15 (33.33%)	5 (31.25%)	3 (42.86%)	0.846

differential agar media that distinguishes it from other urease-producing bacteria. As conventional culture techniques cannot differentiate the two *Ureaplasma* spp. i.e., *U. urealyticum* & *U. parvum* and are sometimes invalidated by the overgrowth of other bacteria.[15] *Mycoplasma* spp. utilizes arginine to produce ammonia through a three-enzyme process, leading to an elevation in the medium's pH. This pH shift is detected by a colour change to red, with phenol red serving as the pH indicator.

Age-wise distribution and prevalence of pathogens

Table 2 provides a comprehensive overview of the age-wise distribution and prevalence of STI pathogens. The sample was collected from women of different age groups and was categorized into six different groups: ≤20, 21-27, 28-34, 35-41, 42-48, and 49-55.

The prevalence of *Ureaplasma* spp. infection was highest in the 28-34-year age group, with 87.88% of culture-positive cases. The lowest occurrence of infections was observed in individuals aged ≤20-year, with all collected samples testing positive for the presence of the bacteria. The prevalence of *N. gonorrhoea* infection remained relatively consistent across different age groups, ranging from 75.76% to 85.71% of culture-positive cases. The age groups with the lowest and highest prevalence were those aged 20 or younger and those aged 42 to 48, respectively. In the case of *Mycoplasma* spp. infection, the prevalence varied across age groups. The highest prevalence was found in the age group of 35 to 41 years, with 33.33% of culture-positive cases. The ≤20-year age group has the lowest prevalence of *Mycoplasma* spp. infection.

Association between Age-specific distribution and prevalence of STI pathogens

The chi-square test was used to assess the link between various pathogens and age groups among tribal women in Madhya Pradesh. Results revealed no statistically significant associations. For *Ureaplasma* spp., the Pearson chi-square test yielded a non-significant value ($\chi^2 = 4.368$, df = 5, p = .498). Similarly, *N. gonorrhoea* ($\chi^2 = 2.024$, df = 5, p = 0.740) and *Mycoplasma* spp. ($\chi^2 = 2.024$, df = 5, p = 0.846) showed no significant association

with age groups. In summary, the p-values for all three pathogen tests exceeded the commonly used threshold for statistical significance (typically set at 0.05 or 0.01), indicating that there was no significant association between pathogen infections (*Ureaplasma* spp., *N. gonorrhoea*, and *Mycoplasma* spp.) and the age group of tribal women in Madhya Pradesh. Consequently, the data did not provide enough evidence to suggest that age group had a significant impact on pathogen infections among tribal women in this study.

Symptoms associated with STI pathogens

Table 3 summarizes correlations between symptoms in individuals diagnosed with *Ureaplasma* spp., *N. gonorrhoea*, and *Mycoplasma* spp. It represents the total reported cases for each symptom and specifies the number associated with each STI pathogen. For example, cloudy/bloody discharge was reported by 71 individuals, with 38 cases linked to *Ureaplasma* spp., 38 to *N. gonorrhoea*, and 17 to *Mycoplasma* spp. Predominantly, lower abdominal pain followed by cloudy/bloody discharge was observed in patients with these STIs. Significance values denote the strength of the symptom-pathogen association, with a p-value less than 0.05 (*) indicating a strong link. This data aids in comprehending symptom patterns for each pathogen, facilitating diagnosis and management.

Antimicrobial susceptibility test

Tables 4, 5 and 6 represent a comparison of the significant levels of various antibiotics categorized in three categories namely sensitive, moderate, and resistant, with corresponding significance values denoted by unique codes. The top three antibiotics from each group were analysed in terms of their sensitivity, along with their respective significance values. Significance values (p-values) as mentioned in table no. 4, 5 and 6 were used to compare their effectiveness and are shown in the Figure 3, 4 and 5 respectively.

Antibiotic sensitivity profiling of *Ureaplasma* spp.

Figure 3 shows results from an antibiogram analysis on *Ureaplasma* spp.

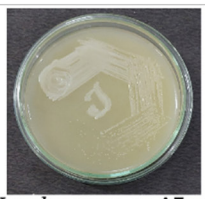
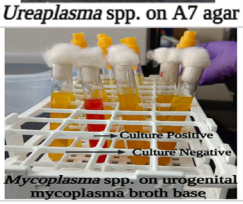
S. N.	Pathogens	Bacterial Growth	Biochemical test
1.	*Neisseria gonorrhoea*	 *N. gonorrhoeae* on TM media	 1. Urease, 2. ONPG, 3. Voges-Proskauer, 4. Oxidase, 5. Catalase, 6. Nitrate reduction, 7. Glucose, 8. Maltose, 9. Lactose, 10. Sucrose, 11. Fructose, 12. Mannose
2.	*Ureaplasma* spp.	 *Ureaplasma* spp. on A7 agar	Typical *Ureaplasma* spp. colonies were identified on A-7 agar (differential agar media) to confirm that the colour change was caused by *Ureaplasma* spp. and not by other urease-producing bacteria
3.	*Mycoplasma* spp.	 *Mycoplasma* spp. on urogenital mycoplasma broth base	*Mycoplasma* spp. utilizes arginine to produce ammonia through a three-enzyme process, leading to an elevation in the medium's pH. This pH shift is detected by a colour change to red, with phenol red serving as the pH indicator.

Figure 2. Pathogen Identification and Confirmatory Analysis

isolates, revealing their antibiotic susceptibility. Antibiotics (ciprofloxacin, norfloxacin, levofloxacin, ofloxacin, tetracycline, doxycycline, azithromycin, erythromycin, roxithromycin) were used for AST, and sensitivity was assessed through the zone of inhibition measurements (Table 1). The figure illustrates varying sensitivity, with ciprofloxacin (34%), doxycycline (29%), and tetracycline (27%) being the most effective, and erythromycin (74%), ofloxacin (69%), roxithromycin (64%) being most resistant. Valuable insights from Figure 3 aid healthcare professionals in prescribing antibiotics for *Ureaplasma* infections.

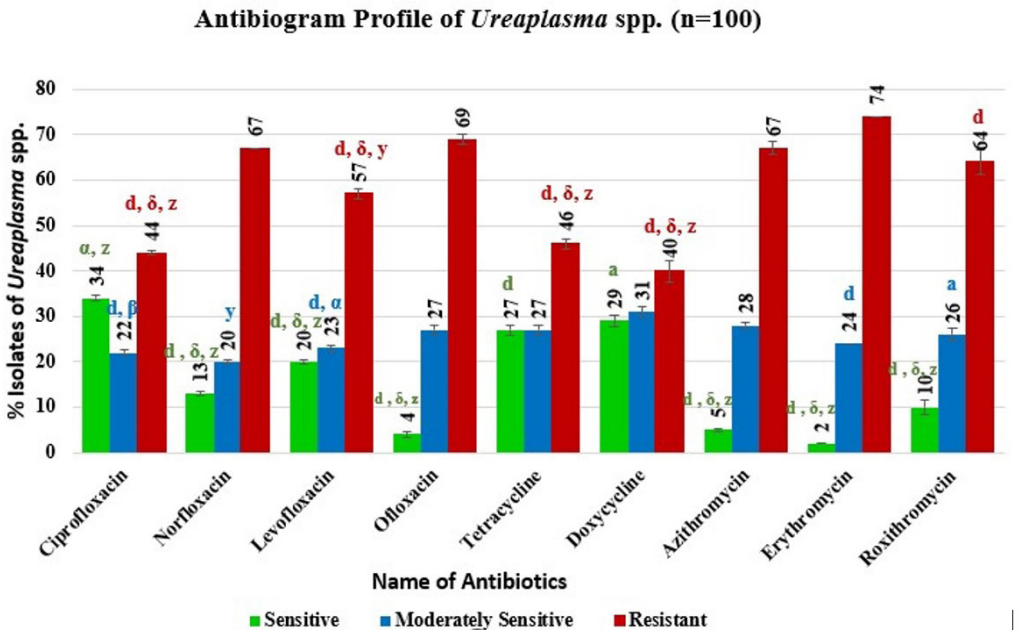


Figure 3. Antibiogram Profile of *Ureaplasma* spp. (n=100)

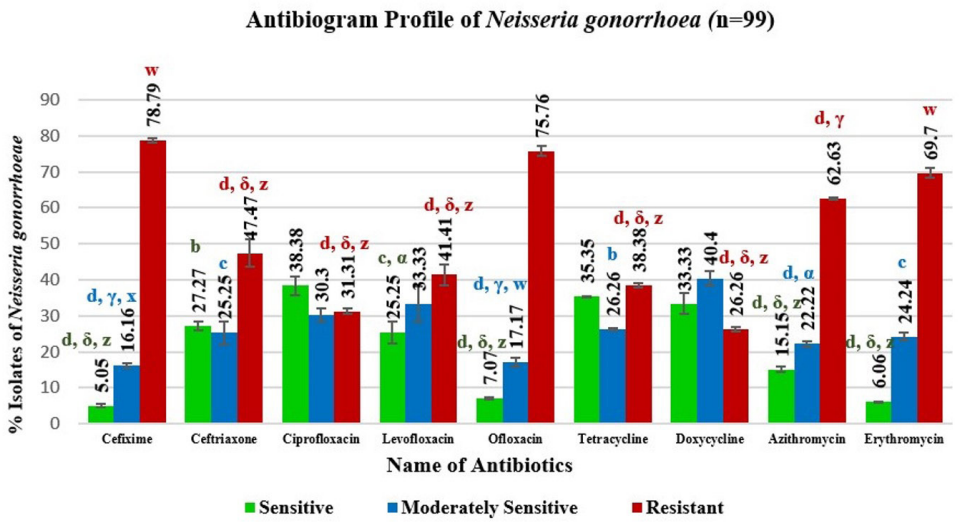


Figure 4. Antibiogram Profile of *Neisseria gonorrhoea (*n=99)

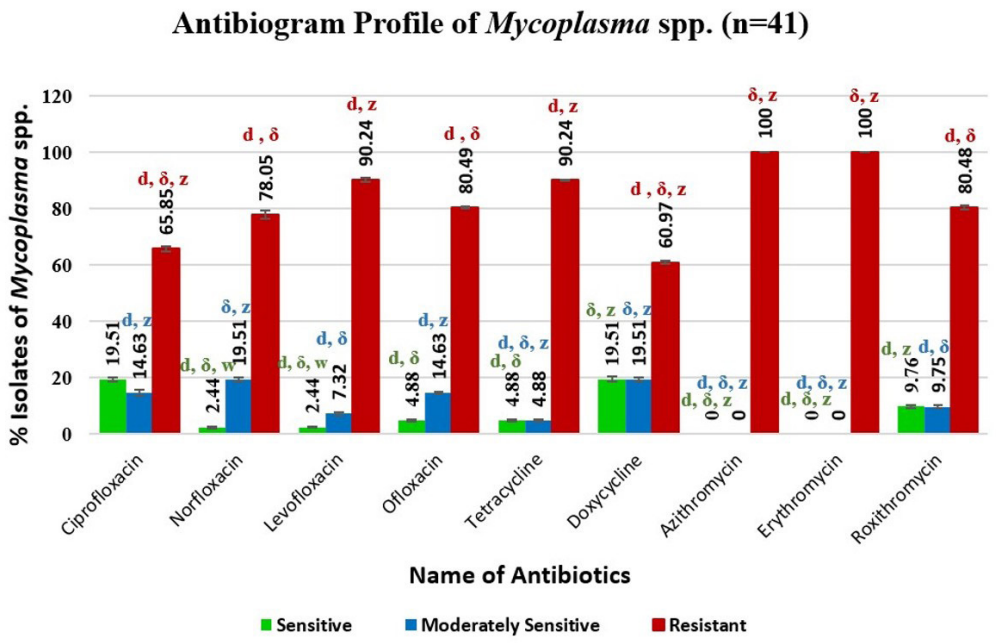


Figure 5. Antibiogram Profile of *Mycoplasma* spp. (n=41)

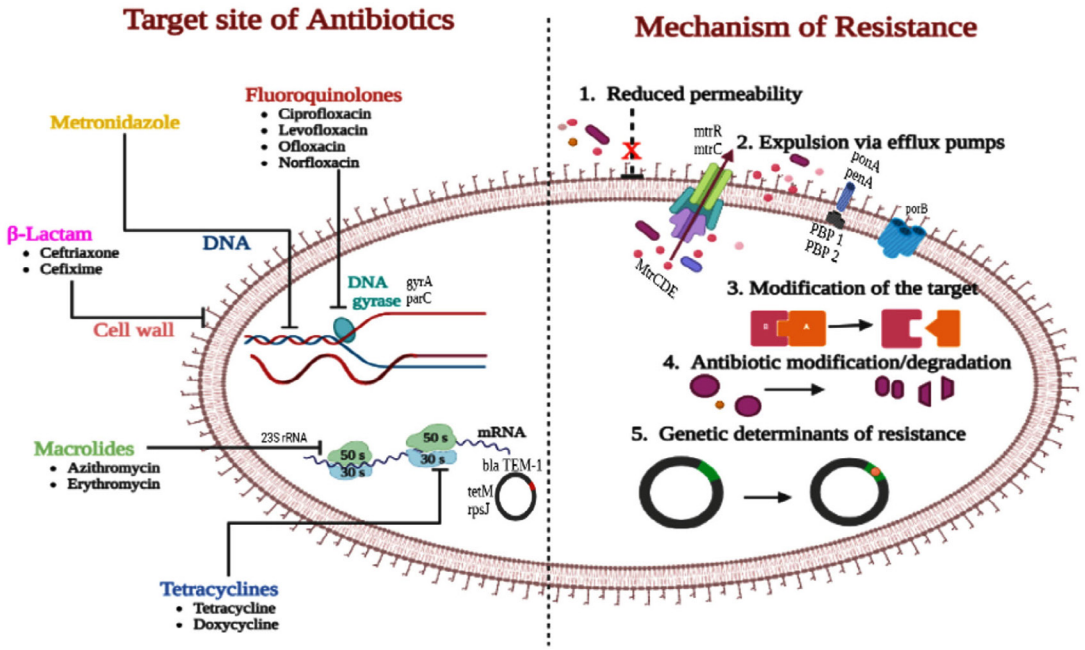


Figure 6. Site of action of some antibiotics & mechanism of resistance[35]

Table 3. Comparative Analysis of Symptoms in STI Pathogens

Symptoms	Total (n=209)	Culture Positive (n=126)	*Ureaplasma* spp.	*Neisseria gonorrhoea*	*Mycoplasma* spp.
Cloudy/bloody discharge	71 (33.97%)	47 (37.30%)	38 (80.85%)	38 (80.85%)	17 (36.17%)
Burning Micturition	38 (18.18%)	22 (17.46%)	12* (54.54%)	13 (59.09%)	10 (45.45%)
Yellow/Green discharge	11 (5.26%)	7 (5.56%)	6 (85.71%)	7 (100%)	1 (14.28%)
Painful intercourse	6 (2.87%)	4 (3.17%)	3 (75%)	3 (75%)	3 (75%)
Strong vaginal odour	37 (17.70%)	24 (19.05%)	17 (70.83%)	19 (79.17%)	8 (33.33%)
Vaginal Itching/Irritation	57 (27.27%)	36 (28.57%)	28 (77.78%)	27 (75%)	16* (44.44%)
Weight loss	1 (0.48%)	0	0	0	0
Lower abdominal pain	108 (51.67%)	62 (49.21%)	42* (67.74%)	44* (70.97%)	18 (29.03%)
Abnormal Menstruation	30 (14.35%)	23 (18.25%)	18 (78.26%)	17 (73.91%)	12* (52.17%)

*= p <0.05 represents significant association between symptoms and pathogens

Table 4. Antibiotic Susceptibility of *Ureaplasma* spp. at different significance levels

Sig.	CIP	DO	TE	DO	AZM	TE & OF	E	OF	AZM & NX
P<0.05	a	α	w	a	α	w	a	α	w
P<0.01	b	β	x	b	β	x	b	β	x
P<0.005	c	γ	y	c	γ	y	c	γ	y
P<0.001	d	δ	z	d	δ	z	d	δ	z
		Sensitive			Moderate			Resistant	

Antibiotic sensitivity profiling of *Neisseria gonorrhoea*

Figure 4 displays the antibiogram of *N. gonorrhoea*, showing the bacteria's sensitivity, moderate sensitivity, and resistance to common antibiotics. Ciprofloxacin, a DNA gyrase inhibitor, exhibits the highest sensitivity at 38.38%, followed by tetracycline and doxycycline. Levofloxacin, ceftriaxone, and tetracycline also demonstrate relatively higher sensitivity (25.25% to 27.27%). The bacteria show significant resistance to cefixime (78.79%), ofloxacin (75.76%), and erythromycin (69.7%), with azithromycin also showing notable resistance (62.63%). These findings offer insights into antibiotic effectiveness against *N. gonorrhoea*.

Antibiotic sensitivity profiling of *Mycoplasma* spp.

Figure 5 summarizes the AST profile of *Mycoplasma* spp. Notably, azithromycin and erythromycin exhibit 100% resistance, while levofloxacin, tetracycline, and ofloxacin show high resistance (80.49% to 90.24%). Conversely, doxycycline and ciprofloxacin are the most effective antibiotics, with a sensitivity rate of 19.51%. Continuous research and surveillance are essential to monitor evolving resistance profiles and guide appropriate antibiotic therapy for *Mycoplasma* spp.

Resistance to multi-drug

AST results reveal MDR in all three STI pathogens across 9 antibiotics. *N. gonorrhoea* exhibits a 56.57% MDR rate (56/99 cases), *Ureaplasma* spp. at 47% (47/100 cases), and *Mycoplasma* spp. with an 80.49% MDR rate (33/41 cases). These percentages signify the resistance of strains within each pathogen to multiple drugs. The analysis underscores a troubling trend of rising resistance in these pathogens, posing challenges for effective medical intervention.

DISCUSSION

Concerns regarding the high prevalence of STIs remain crucial, as these infections can lead to various individual and community health complications. Furthermore, the emergence of drug-resistant organisms and the substantial economic burden associated with STIs underscore

Table 5. Antibiotic Susceptibility of *Neisseria gonorrhoea* at different significance levels

Sig.	CIP	TE	DO	DO	LE	CIP	CFM	OF	E
P<0.05	a	α	w	a	α	w	a	α	w
P<0.01	b	β	x	b	β	x	b	β	x
P<0.005	c	γ	y	c	γ	y	c	γ	y
P<0.001	d	δ	z	d	δ	z	d	δ	z
		Sensitive			Moderate			Resistant	

Table 6. Antibiotic Susceptibility of *Mycoplasma* spp. at different significance levels

Sig.	CIP & DO	RO	TE & OF	DO & NX	OF & CIP	RO	AZM & E	TE & LE	OF
P<0.05	a	α	w	a	α	w	a	α	w
P<0.01	b	β	x	b	β	x	b	β	x
P<0.005	c	γ	y	c	γ	y	c	γ	y
P<0.001	d	δ	z	d	δ	z	d	δ	z
		Sensitive			Moderate			Resistant	

the need for ongoing efforts to enhance disease management.[18] The result of this study showed that about 79.36% of symptomatic women harboured *Ureaplasma* spp. This rate is slightly higher to the prevalence of *Ureaplasma* spp. in China was 54.5%,[19] to that in an Italian study (23%) and the rate obtained in Croatian study (34%) as mentioned by Karim et al.[20] Highest prevalence had seen between the age group 35-41 years. Based on the bacterial culture result, the prevalence of *N. gonorrhoea* in the Anuppur district is 78.57% while *Mycoplasma* spp. possessed a 32.54% prevalent rate. The overall highest prevalence for all three pathogens occurred in the 35-41 age group, while the second highest prevalence for all three pathogens was observed in the 28-34 age group. A similar prevalence age group (30-34 years followed by the 35-39 years) pattern in women was observed in a study conducted in the tribal population of central India.[1]

This suggests that individuals in their mid to late thirties and early forties may be particularly susceptible to these infections, possibly due to certain behavioural or biological factors prevalent in this age range. The finding might reflect a lack of awareness about safe sexual practices and the importance of regular screening for sexually transmitted infections in their mid to late thirties and early forties.

Antibiotics are generally biologically active, inhibiting protein, nucleic acid, and cell wall synthesis, as well as DNA replication and cell division.[22] Misuse of these drugs has led to the proliferation of antibiotic resistance in many bacterial strains.[12] The pathogen *N. gonorrhoeae* has a remarkable potential to acquire resistance to clinically utilized antimicrobial medicines within 10-20 years.[23] New medications replace antibiotics that have lost their potency, but new strains emerge with new resistance determinants; this problem affects all antibiotic classes.[13] Single-dose antibiotics effectively treat gonococcal and non-gonococcal sexually transmitted infections, but rising resistance, as reported,[24] poses a growing threat. Antibiotic resistance has surged over the past few decades, fuelled by overuse and abuse, exacerbated by factors like international travel and commerce. This crisis stems from a long history of neglect despite repeated warnings from researchers and clinicians since the early 1960s.[25]

N. gonorrhoea rapidly develops resistance to newly introduced antimicrobials for gonorrhoea treatment within 1-2 decades, showcasing its persistent adaptability since the antimicrobial era's inception in the 1930s.[26] This study found high resistance of *N. gonorrhoeae* to commonly prescribed drugs, notably 78.79% for cefixime, 75.76% for ofloxacin, 69.7% for erythromycin, and 62.63% for azithromycin.

The absence of a cell wall makes *Ureaplasma* spp. and *Mycoplasma* spp. inherently resistant to all medications that target the

cell wall, including β-Lactam.[27] *Ureaplasma* spp. exhibit antibiotic resistance via distinct mechanisms like Macrolide resistance entails 23S rRNA gene mutations, fluoroquinolone resistance stems from parC gene mutations in quinolone resistance-determining regions, and tetracycline resistance is linked to acquiring the TetM gene on Tn916-like mobile elements.[28] In a Tunisian study, *Ureaplasma* spp. isolates showed resistance to ciprofloxacin and erythromycin, with intermediate resistance to azithromycin. Ofloxacin resistance was noted in 73.27% of isolates, and levofloxacin resistance in 17.82%. Additionally, 37.62% of isolates exhibited resistance to tetracycline.[29] AST result of *Ureaplasma* spp. of this study showed a resemblance to the work done by Boujemaa and co-workers.

M. hominis exhibits intrinsic resistance to C14 and C15-membered macrolides, including erythromycin, and azithromycin. However, promising antibiotics that display excellent efficacy against *M. hominis* are doxycycline and tetracycline.[30] Among the antibiotics tested in this study, azithromycin and erythromycin show the highest resistance, with 100% of *Mycoplasma* spp. being resistant to it. Levofloxacin, tetracycline, and ofloxacin also exhibit high resistance rates, ranging from 80.49% to 90.24%. Some previous studies also documentation of 100% erythromycin resistance.[31,32] The resistance mechanism probably involves a target alteration in the DNA gyrase and topoisomerase IV subunits.[33] Doxycycline and ciprofloxacin with a sensitivity rate of 19.51% can be considered as a drug of choice for treating genital *Mycoplasma* infection. Tetracyclines showed only moderate sensitivity. Tetracycline resistance due to the acquisition of tetM gene is widely reported.[33] Knowledge of local patterns of antimicrobial resistance facilitate clinicians to choose best treatment options for the patients. Most of the known resistance mechanisms, including antibiotic inactivation, drug binding site modification, membrane permeability decrease, and enhanced drug efflux.[34] The site of action and mechanism of resistance of antibiotics is depicted in Figure 6.

As suggested by Gonzalez and his colleagues in 2009 that the high rates of poverty, income inequality, unemployment and low educational attainment make it more difficult for individuals to protect their sexual health.[36] Tribal females need special attention for the prevention of STIs as in tribal areas, there is little or no access to the health delivery system. also, many researchers have found that tribal societies have a system of multiple sex partners and unprotected sex.

CONCLUSION

The research provides valuable insights regarding the prevalence and antibiotic resistance pattern of STI pathogens in District Anuppur. The increased frequency of STI pathogens may be due to polygamy, endogamy and little or no access to the health delivery system. STI pathogens also acquire resistance to the currently recommended antibiotics either due to the exhibition of an extraordinary ability of phenotypic and genotypic variation or due to misuse and overuse of drugs. In view of the present findings, an urgent need for appropriate interventions is required to address the growing threat of STI pathogens and antimicrobial resistance in this region.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

PS conceptualized and designed the study. VH and SX collected the samples from district hospital Anuppur. SS and NJ helped to facilitate

the swab sample collection from Birsamunda Government Medical College, Shahdol. VH perform bacterial culture of pathogens, analysed the sample data on the Age-wise and symptoms-wise prevalence of STI patients. J isolated and identified the pathogens, performed antimicrobial susceptibility test experiments. RS analysed the results. J wrote the manuscript. PS and RS edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This work was approved by the Institutional Ethical Committee, Indira Gandhi National Tribal University, Amarkantak, wide Ref. No., IGNTU/IEC/01/2019, dated 11/05/2019 and Govt. Medical College Shahdol wide Ref. no. IERC/22/06/001, Dated 16/06/2022.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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