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# Antagonistic Activities of Lactic Acid Bacteria against Organisms Implicated in Urogenital Infections

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**ABSTRACT**

Urogenital infections are common infectious state that may be sexually or non sexually transmitted. There is an increasing dilemma in treatment options due to bacterial resistance to antibiotics. This study was carried out to determine the ability of Lactic Acid Bacteria (LAB) to inhibit the growth of two uropathogens (bacterium *Neisseria gonorrhea* and yeast *Candida albicans)invitro*

The antibiotic resistance patterns of two strains of *Neisseria gonorrhea* to ten antibiotics were tested by disc diffusion method. The inhibition of the growth of *Neisseria gonorrhea* UCH STC 2021 and *Candida albican*o UCH STC 2023 by viable LAB were tested by overlay method and the cell free supernatant of LAB were tested against 4 strains of *Candida albicans* and two strains of *Neisseria gonorrhea* by agar well diffusion assay.

*Neisseria gonorrhea* UCH STC 2022 was only sensitive to ciprofloxacin while resistance was observed to the remaining nine tested antibiotics while *Neisseria gonorrhea* UCH STC 2021 was sensitive to ciprofloxacin, ofloxacin and amoxicillin with resistance observed in the remaining seven antibiotics. *Neisseria gonorrhea* UCH STC 2021 was sensitive to 75% of the tested LAB while *Candida albicans* UCH STC 2023 was sensitive to 55% of the tested LAB. All the uropathogenic strains were resistant to all the tested cell free LAB supernatant with no observed zone of inhibition.

The result from this study shows that LAB cab be an option for treatment of antibiotic resistant bacterial uropathogens and yeast *Candida albicans*.

**KEYWORDS**: Therapeutic agent, Resistance, *Candida albicans, Neisseria gonorrhea*

## INTRODUCTION

The microbes that inhabit the vagina play a major role in illnesses of the host, including bacterial vaginosis, yeast vaginitis, cancer, and sexually transmitted diseases, such as human immunodeficiency virus infection and gonorrhea, as well as in the maintenance of a healthy tract [1]. Gonorrhea is an acute Sexually Transmitted Infection (STI) which in nature affects only human. It is caused by bacterium *Neisseria gonorrhea*. In males, infection may be asymptomatic or may involve arthritis prostatitis and epididymitis with painful urination and a yellowish mucopurulent discharge while in female, infection may be asmtomatic or there may be a purulent vagina discharge. The infectious situations related to the female genital tract are more and more frequent by their direct relationship to the increase of STI which include those produced by bacteria, virus, fungi and parasites.

Yeast vaginitis is an urogenital infections not caused by sexual transmission. 8% of women reported 4 or more episodes during a 1-year period with propensity for recurrence. Yeast vaginitis is a very common problem, estimated to affect around 1:5 black American women and close to 1:10 white women during any given two month time frame, with 1:12 reporting four or more episodes per year [2]. The intestine is the main source of the infecting fungal organisms, and overgrowth in the vagina can follow disruption of the normal flora such as with the use of broad spectrum antibiotic treatment. The clinical picture of yeast vaginitis is generally clear—

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the development of a white vaginal discharge characterised by its malodorous, non-homogenous caseous appearance, accompanied with vaginal itch and irritation, and evidence of vaginal inflammatory reaction. Thetre are anti yeast medication but, there is usually reoccurence While *Candida albicans* is the major cause of yeast vaginitis infections (around 85%), other yeast such as *Candida glabrata*, *Candida krusei*, and *Candida tropicalis* also infect the host.

Infectious diseases account for approximately one half of all death in tropical countries especially African countries. Incidence of epidermics due to drug resistant microorganisms and the emergence of unknown disease causing micro-organism pose enormous public health concern. Hence, the need for new and effective antimicrobial agents without the additional problem of drug resistance. Lactic Acid Bacteria (LAB) are normally isolated from fermented foods while some species are normal flora of human gastrointestinal tract. The industrial importance of the LAB is further evidenced by their Generally Regarded As Safe (GRAS) status, due to their ubiquitous appearance in food and their contribution to the healthy microflora of human mucosal surfaces.. The ability of LAB to inhibit various pathogens is well documented. [3,4]. Therefore, the objectives of this work are determination of resistance of a bacterial genus implicated in urogenital infections to antibiotics and screening for antimicrobial activities of LAB against a bacterial and fungal uropathogens.

## MATERIALS AND METHODS

### Bacterial Srains

Twenty strains of LAB were isolated from different indigenous fermented dairy foods and identified as species of *Lactobacillus fermentum, L. brevis, L. plantarum, Lactococcus lactis* and *Streptococcus durans* as previously described by Adeniyi *et al.*, [5] Organisms implicated in urogenital infections were isolated and collected from Sexually Transmitted Clinic (STC) of University College Hospital, (UCH), Ibadan, Nigeria. They are *Candida albican* UCH STC 2023, *Candida albican* UCH STC 2024, *Candida albican* UCH STC 2025, *Candida albican* UCH STC 2026 and *Neisseria gonorrhea* UCH STC 2021, *Neisseria gonorrhea* UCH STC 2022.

### Antibiotic Susceptibility Testing

Susceptibility testing for two strains of *Neisseria gonorrhea* were performed using disc diffusion method of Kirby-Bauer according to National Committee for Clinical Laboratory Standards guidelines. The antibiotic sensitivity disc consists of different antibiotics namely Colistin 25 µg, Amoxycillin 25 µg, Cotrimoxazole 25 µg, Augmentin 30 µg, Nalidix acid 30 µg, Ciprofloxacin 10 µg, Gentamicin 10 µg, Ofloxacin 30 µg, Tetracycline 30 µg and Nitrofuranton 200 µg. Each antibiotic disc was placed on Mueller Hinton agar plates seeded with *Neisseria gonorrhea*. The plates were incubated at $37^0$C for 24 h. The diameter of the zones of inhibition were measured and resistance was determined according to the reference zone diameter interpretative standard of NCCLS.

### Antimicrobial Assay

### Overlay Method

An overlay method [6] was used to determine the ability of 20 strains of LAB of the following species- *Lactobacillus fermentum, L. brevis, L. plantarum, Lactococcus lactis* and *Streptococcus durans* to inhibit the growth of *Neisseria gonorrhea* UCH STC 2021 and *Candida albican* UCH STC 2023. It was performed using MRS agar plates on which a loopful of LAB in MRS broth was inoculated as approximately 2cm long lines. After incubation for 24 h at 37°C in a microaerophilic environment, the plates were overlaid with approximately $10^5$ CFU/ml of the indicator pathogens (*Neisseria gonorrhea* and *Candida albican*) vehiculated in 10 ml of Mueller Hinton soft agar and Sabouraud Dextrose soft agar (0.7% agar) respectively. The plates overlaid with *Neisseria gonorrhea* were incubated at 37°C for 24 h in an aerobic environment while the plates overlaid with *Candida albican* were incubated at room temperature for 24 h in an aerobic environment . Finally, the plates were examined for clear zones of inhibition around the LAB streaks.

### Antagonistic Activities of LAB Metabolites in Cell Free Supernatant

The following LAB strains- *Lactobacillus fermenti* HW7*, L. brevis* M5*, L. plantarum* N2*, Lactococcus lactis* K3and *Streptococcus durans* K4 that showed clear zones of antimicrobial activity against tested uropathogens were grown in MRS broth overnight at 37°C. The culture was centrifuged at 12,000 g for 10 min at 4°C. The antimicrobial activity of the cell-free supernatant was determined by an agar well diffusion assay. Aliquots (30 µl) of the supernatant were placed in wells (7-mm diameter), cut in cooled Mueller Hinton plates seeded with the indicator organisms (*Neisseria gonorrhea* UCH STC 2021 and *Neisseria gonorrhea* UCH STC 2022.) and Sabouraud Dextrose agar seeded with *Candida albican* UCH STC 2023, *Candida albican* UCH STC

2024, *Candida albican* UCH STC 2025 and *Candida albican* UCH STC 2026. The plates were incubated at 37°C and room temperature respectively for 24 h in an aerobic environment and examined for clear zones of inhibition around the wells

## RESULTS AND DISCUSSION

The antibiotic resistance patterns of two strains of *Neisseria gonorrhea* were tested by disc diffusion and the results are shown in Table I. *Neisseria gonorrhea* UCH STC 2022 was only sensitive to Ciprofloxacin while resistance was observed to the remaining nine tested antibiotics while *Neisseria gonorrhea* UCH STC 2021 was sensitive to Ciprofloxacin, Ofloxacin and Amoxicillin with resistance observed in the remaining seven antibiotics.

The inhibition of the growth of *Neisseria gonorrhea* UCH STC 2021 and *Candida albican*o UCH STC 2023 by viable LAB were tested. One or both of the sexually transmitted pathogens were sensitive to 17 LAB. Both pathogens were sensitive to 5 strains of *Lactobacillus fermenti,* 2 strains of *Lactobacillus brevis* and 2 strains of Lactobacillus *plantarum*. (Table II). *Neisseria gonorrhea* UCH STC 2021 was sensitive to 75% of the tested LAB while *Candida albicans* UCH STC 2023 was sensitive to 55% of the tested LAB.

The cell free supernatants of LAB were tested against 4 strains of *Candida albicans* and two strains of *Neisseria gonorrhea*. It was observed that all the pathogenic strains were resistant to all the tested cell free supernatant with no observed zone of inhibition.

## DISCUSSION

A lot of studies have been reported on the antagonistic activity of LAB against organisms implicated in urogenital infections [5,7]. The high antimicrobial potentials displayed by LAB against uropathogens observed in this study is in correlation with previous studies in which species of LAB were selected due to their antimicrobial activity against uropathogens [5,8]. The correlation between a healthy vaginal tract, as defined by lack of symptoms and signs of diseases with dominance of lactobacilli supports the belief that these commensals play a major role in preventing certain types of vaginal infections. [1]. Orally administered lactobacilli reach the vagina via the anus and the perineal and vulval skin, as do pathogens, irrespective of hygiene. [9].

*Neisseria gonorrhea* UCH STC 2021 (indicator *Neisseria gonorrhea*) was only sensitive to three out of ten antibiotics used against it. This agrees with Bruce *et al*., [10] who reported gonococcal resistance to penicillin, tetracycline, spectinomycin and other antimicrobial agents. However, as observed in this study, the organism was sensitive to 75% of viable LAB used against it.

Reid *et al.* [11] reported that the antimicrobial activities of LAB could be due to the production of antimicrobial compounds such as lactic and acetic acid, hydrogen peroxide, bacteriocins as well as compounds not yet identified. These metabolites are soluble in liquid broth and therefore can be detected in supernatant of LAB cultures [12]. The LAB's cell free supernatant containing metabolites have no effect on uropathogens used in this study. However, when their viable cells were used against the indicator uropathogens, sensitivity were shown by *Candida albicans* UCH STC 2023 to 55% of viable LAB used against it. This finding agrees with Reid *et al*., [8] who reported that daily oral ingestion of some strains of specific Lactobacilli significantly improve the vagina flora lowering the yeast and coliform count. Likewise, sensitivity were shown by *Neisseria gonorrhea* to 75% of viable LAB used against it. It can therefore be inferred from this study that that it is only antimicrobial producing viable LAB that can inhibit the growth of uropathogens used in this study but not their metabolites contrary to the report of Adeniyi *et al*,, [5] who reported antagonism of the growth of uropathogens by cell free supernatant of LAB containing metabolites. The findings from this study imply that there must be an interaction between viable LAB and the studied uropathogens before inhibition of growth can occur.

In conclusion, certain LAB have been shown in this study to inhibit the growth of *Neisseria gonorrhea* and *Candida albicans* implicated in urogenital infections. The uropathogens which showed great resistance in varying degree to antibiotics were sensitive to antimicrobial producing viable LAB. These LAB could be used as prophylactic and therapeutic agent for urogenital infectionss since cases of urogenital infections are increasing in Nigeria. The ingestion of LAB as probiotics is a feasible option for the treatment of urogenital infection especially in cases of resistance to antibiotics.

**Table I**: **Antibiotic Sensitivity Profile of strains of *Neisseria gonorrhea***

| | Indicator bacteria | |
|---|---|---|
| Antibiotics | *Neisseria gonorrhea* UCH STC 2021 | *Neisseria gonorrhea* UCH STC 2022 |
| Tetracycline | R | R |
| Colistin | R | R |
| Augmentin | R | R |
| Ofloxacin | S | R |
| Gentamicin | R | R |
| Nalidix acid | R | R |
| Ciprofloxacin | S | S |
| Nitrofuranton | R | R |
| Cotrimoxazole | R | R |
| Amoxycillin | S | R |
| % Resistance | 70 | 90 |

Key: S-Sensitive, R-Resistant

**Table II**: **Sensitivity of *Neisseria gonorrhea* and *Candida albican* to viable lactic acid bacteria**

| | Indicator organism | Micro- |
|---|---|---|
| LAB | *Candida albican* UCH STC 2023 | *Neisseria gonorrhea* UCH STC 2021 |
| *Lactobacillus fermenti* K1 | R | R |
| *Lactobacillus fermenti* HW7 | S | S |
| *Lactobacillus fermenti* W1 | S | R |
| *Lactococcus lactis* K2 | R | S |
| *Lactococcus lactis* K3 | R | S |
| *Streptococcus durans* K4 | R | R |
| *Lactobacillus fermenti* N1 | S | S |
| *Lactobacillus fermenti* HW8 | R | S |
| *Lactobacillus brevis* M5 | R | S |
| *Lactobacillus plantarum* HW8 | S | S |
| *Lactobacillus plantarum* N2 | S | R |
| *Lactobacillus plantarum* N3 | S | S |
| *Lactobacillus fermenti* N4 | R | S |
| *Lactobacillus fermenti* N2 | S | S |
| *Lactobacillus fermenti* N5 | S | S |
| *Lactobacillus fermenti* W3 | S | S |
| *Lactobacillus brevis* M1 | R | R |
| *Lactobacillus brevis* M2 | S | S |
| *Lactobacillus brevis* M3 | S | S |
| *Lactobacillus brevis* M4 | R | S |
| % Susceptibility | 55 | 75 |

Key: S-Sensitive, R-Resistant

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