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# Etiologic Agents and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Urines of Antiretroviral Therapy Users

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**Abstract** The aim of this study was to determine the etiologic agents and the antibiotic susceptibility pattern of *Staphylococcus aureus* isolated among antiretroviral therapy users with asymptomatic urinary tract infections. Urine samples were collected for the diagnostic purpose. Uropathogen bacteria were identified using microbiological conventional methods, and the antibiotic susceptibility test was carried out using disk diffusion method. The predominant microorganisms isolated were *Escherichia coli* (49.6 %) followed by coagulase negative staphylococci (18.4 %) and *Staphylococcus aureus* (14.1%). All *Staphylococcus aureus* identified were resistant to methicillin and multidrug resistant. The results of the present study suggest the use antibiotic susceptibility test before treatment of urinary tract infection in human immunodeficiency virus infected patients.

***Keywords:*** *human immunodeficiency virus, etiologic agents, Staphylococcus aureus, antibiotics, Democratic Republic of Congo*

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## 1. Introduction

In people living with the human immunodeficiency virus (HIV), the opportunistic infections may raise the incidence of HIV and the progression to acquired immunodeficiency syndrome (AIDS)[1]. Literature data have shown that patients with AIDS are predisposed to urinary tract infections (UTI), and the prevalence of UTI in AIDS patients is high compared to the control group [2]. Symptoms include dysuria, frequency pollakiuria, urgency, fever, lumbar pain and haematuria, although many patients are asymptomatic [3,4,5]. The most common bacterial pathogens in HIV-infected patients and which may affect any urogenital organ are *Enterobacteriaceae*, *Staphylococcus*, and *Enterococcus* spp [5,6,7].

In Democratic Republic of Congo (DRC), bacterial opportunistic infections; including UTI in HIV/AIDS infected persons are treatable with broad or narrow spectrum antibiotics without antibiotic susceptibility tests. But the control of UTI constitutes a challenge because of the emergence of multiple antibiotic resistances [5]. In a previous report, we have demonstrated that, *Escherichia coli* (62.5%) followed by *Klebsiella spp* (18.75 %), *Enterobacter spp* (12.5 %), and *Proteus spp* (6.25 %) were the major bacteria isolated from the urine of Kinshasa HIV-infected patients without AIDS [8]. Isolated bacteria were sensitive to cefotaxim, ceftriaxon, and gentamicin and resistant to chloramphenicol, cotrimoxazole, tetracycline, and norfloxacin [8]. Until now, there is no published report on etiologic agents and particularly on antimicrobial susceptibility patterns of *S. aureus* isolated from Congolese ART users with asymptomatic UTI. This study aimed to determine the etiologic agents and the antibiotic susceptibility pattern of *S. aureus* isolated among ART users with asymptomatic UTI.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Bacterial Strains

This study was conducted between March 2014 and June 2015 in the Hôpital de l'Amitié Sino Congolaise, Centre Mère et Enfant in Barumbu and in Centre Bomoyi in Kinshasa. Urine samples from 287 HIV/AIDS patients were collected in these three hospitals and analysed in the

Laboratory of Experimental and Pharmaceutical Microbiology of the Faculty of Pharmaceutical Sciences, University of Kinshasa.

## 2.2. Methods

### 2.2.1. Isolation and Identification of Bacterial Strains

The isolation of pathogen susceptible bacteria was done by culivating the 287 urine samples in Hektoen, Mac Conkey and Mannitol Salt Agars media (Liofilchem, Roseto degli Abbruzzi, Italy). The numeration of bacterial colonies was performed using plates of Cystein Lactose Electrolyte Deficient (CLED) agar (Liofilchem, Roseto degli Abbruzzi, Italy) which were incubated at 37°C for 24 hours. Cultures were considered positive when $10^5$ Colony Forming Units (CFU) were counted.

Isolated strains of *Enterobacteriaceae* were identified using microbiological conventional methods including Gram staining, oxydase tests, indole and urease production, citrate utilization, hydrogen sulphide, gas production and fermentation of sugars, and lysine decarboxylase (L.D.C.), ornithine decarboxylase (O.D.C.) and arginine dihydrolase (A.D.H.) tests. The strains of *Staphylococcus* was detected using Gram staining and catalase reaction. The identification of *S. aureus* strains was performed with latex agglutination test (Pastorex Staph-Plus, BioRad, Marnes-la-Coquette, France) and DNase test. All staphylococcal strains, negative for latex agglutination and DNase tests, were considered as coagulase negative staphylococci.

### 2.1.2. *S. aureus* Antibiotic Susceptibility Tests

The isolated *S. aureus* strains were tested for their antimicrobic susceptibility by the diffusion method on Mueller Hinton Agar using the following antibiotic disks (Liofilchem, Roseto degli Abruzzi, Italy): nalidixicacid (75 µg), ciprofloxacin (5 µg), gentamicin (30 µg), kanamycin (30 µg), erythromycin (15 µg), imipenem (10 µg), cotrimoxazole (25 µg), norfloxacin (10 µg) and vancomycin (30 µg). Test for methicillin resistance was performed by diffusion method on Mueller Hinton agar (Liofilchem, Roseto degli Abbruzzi, Italy) with 4 % NaCl using oxacillin (1 µg). Evaluation of the results was done according to the criteria of Clinical Laboratory Standards Institute (CLSI) [9]. *S. aureus* ATCC 25923 was used for quality control.

# 3. Results

## 3.1. Characteristics of the Patients

Two hundred and eighty-seven HIV/AIDS patients under ART were enrolled. The age range was 16-70 years. The female population predominated as there were 75 males (26.0%) and 212 (74.0%) females (Table 1).

**Table 1. Characteristics of participants**

| Age group (years) | Male (%) | Female (%) | Total (%) |
|---|---|---|---|
| 16 - 26 | 5 (1.7) | 10 (3.5) | 15 (5.4) |
| 27 - 37 | 28 (9.7) | 39 (13.5) | 67 (23.2) |
| 38 - 48 | 17 (5.9) | 60 (20.8) | 77 (26.7) |
| 49 - 59 | 20 (6.9) | 100 (34.7) | 120 (41.6) |
| 60 - 70 | 5 (1.7) | 3 (1.0) | 8 (2.8) |
| Total | 75 (26.0) | (212) (74.0) | (287) (100) |

### 3.2.4. Prevalence of UTI and Uropathogens Isolated

The major organisms isolated from urine samples are shown in Table 2. Samples from 163 (56.7 %) patients were significant for UTI (bacterial count $\geq 10^5$ CFU / ml); the other 124 cultures were sterile or had no significant bacteriuria. *E. coli* was the most common pathogen isolated (49.6%), followed by CNS (18.4 %) and *S. aureus* (14.1%), whereas *Enterobacter* species and *Klebsiella oxytoca* accounted for 8.5 % and 4.2 % of all isolates, respectively, and *Salmonella* and *Proteus vulgaris* species each for 1.8 %.

**Table 2. Organisms isolated on culture**

| Organisms | No (%) |
|---|---|
| *Escherichia coli* | 81 (49.6) |
| *Staphylococcus aureus* | 23 (14.1) |
| Coagulase negative Staphylococci (CNS) | 30 (18.4) |
| *Enterobacter spp.* | 14 (8.5) |
| *Klebsiella oxytoca* | 7 (4.2) |
| *Klebsiella pneumonia* | 2 (1.2) |
| *Salmonella spp* | 3 (1.8) |
| *Proteus vulgaris* | 3 (1.8) |
| Total | 163 (100) |

## 3.3. Antibiotic Susceptibility of *S. aureus* Strains

The antibiotic sensitivity patterns of *S. aureus* from this study showed that all 23 strains were resistant to oxacillin and nalidixic acid. More than 60 % of *S. aureus* strains were resistant to cotrimoxazole, erythromycin and vancomycin, whereas about 56 % of them were resistant to ciprofloxacin, norfloxacin and kanamycin. High rate resistance (43.5 %) of *S. aureus* strains against to imipenem and gentamicin was also observed (Table 3).

**Table 3. Antibiotic susceptibility patterns of *S. aureus* isolates**

| Antibiotics | Susceptible (%) | Resistant (%) |
|---|---|---|
| Kanamycin | 10 (43.5) | 14 (56.5) |
| Gentamicin | 13 (56.5) | 10 (43.5) |
| Ciprofloxacin | 10 (43.5) | 13 (56.5) |
| Norfloxacin | 10 (43.5) | 13 (56.3) |
| Nalidixic acid | 0 (0.0) | 23 (100.0) |
| Erythromycin | 3 (13.0) | 20 (87) |
| Vancomycin | 9 (39.0) | 14 (61.0) |
| Oxacillin | 0(0.0) | 23(100.0) |
| Imipenem | 13(56.5) | 10(43.5) |
| Cotrimoxazole | 3(13.0) | 20(87.0) |

# 4. Discussion

The results of the present study showed a high prevalence (56.7 %) of UTI in HIV/AIDS patients under ART. This rate was higher than those observed in previous studies conducted in Ethiopia [7] and in Nigeria [10] with prevalence rates of 12 % and 25.3 % respectively. Factors which may contribute to the high incidence of UTI could be the difference in the immune responses of the patients, the stage of HIV/AIDS disease, the interruption of ART, the no protected sexual activity, or the failure of hygiene practices. All these risk factors

were not analyzed in this study. We observed in this work that *E. coli was* the predominant uropathogen bacteria, followed by CNS and *S. aureus* in HIV/AIDS patients under ART, in contrast to the studies done in Benin, Nigeria [11] and Calabar City [10] in which *S. aureus* was the predominant isolated bacterium in urine of those patients, followed by *E. coli*. However, our findings were in accord with the results reported in previous studies from Ethiopia and Nigeria [2,7].

Enterobacterial strains were the most common isolated bacteria with 110 cases (64.5 %), whereas *Staphylococcus* species accounted for 53 cases (32.5%). In general, etiologic agents isolated from UTI are in agreement with commonly isolated bacteria in other African studies [6,7,5], with the exception of *Salmonella* species. Nontyphoidal *Salmonella* (NTS) is an unusual cause of UTI, but responsible for causing various types of human infections. Our observation was in accord with that obtained previously [12] which reported that a NTS strain was responsible for UTI in HIV patients. The 3 strains of *Salmonella spp*. isolated from women, are the first documented cases of NTS UTI in HIV/AIDS patients from Democratic Republic of Congo.

The investigation of the antibiotic susceptibility showed that all strains of *S. aureus* isolated from HIV/AIDS ART users were methicillin resistant. Methicillin resistant *S. aureus* (MRSA) infections in HIV/AIDS infected patients are considered The majority of *S. aureus* strains isolated from HIV/AIDS patients under ART were resistant to vancomycin and to other commonly used antibiotics, such as nalidixic acid, cotrimoxazole, erythromycin, ciprofloxacin, norfloxacin and kanamycin. The MRSA strains were generally multidrug resistant. Study done in Jimma University Specialized Hospital, Ethiopian [7] showed that *S. aureus* from urine of ARTS users were resistant to some antibiotics tested in this study such as nalidixic acid and cotrimoxazole and sensitive to norfloxacin and erythromycin. It was also important to note in our study the increasing rate of resistance of *S. aureus* strains against imipenem. Various factors could be responsible of the emergence of multiresistant strains of MRSA, such as the empirical and irrational use of antibiotics in community and hospitals.

# 5. Conclusion

The present study revealed a high prevalence of UTI in HIV/AIDS patients under ART. *E. coli*, followed by CNS and *S. aureus* were the predominant uropathogen agents. All isolated MRSA strains were multidrug resistant and this could lead to critical illness in HIV-infected patients. Therefore, it must be recommended to perform the antibiotic susceptibility test prior to UTI treatment.

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