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Research Article

Cotrimoxazole Resistance Profile of Bacterial Strains Isolated from Urine Samples in Children Born to HIV Positive Mothers Compared to HIV Non-exposed Children

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Abstract

Background: WHO has recommended a systematical administration of cotrimoxazole to children born to HIV-positive mothers to limit or reduce the occurrence of opportunistic infections. This systematic use of cotrimoxazole is associated with the evident risk of increased antibiotic resistance. We aimed to determine the prevalence of cotrimoxazole resistance and the resistance profile of strains isolated from urine samples of children born to HIV-positive mothers compared to HIV non-exposed, cotrimoxazole naïve children.

Methods: Children presenting with urinary tract infection were enrolled and grouped as cotrimoxazole treated or naïve. Bacteria isolates from urine samples of these children and were identified using standard microbiology techniques. Antibiotic susceptibility testing was performed using the disc diffusion technique. Data were analyzed using the Statistical Package for Social Science (SPSS) version 20.

Results: A total of 137 children under 15 years of age were enrolled. Of these, 98 were not HIV-exposed and cotrimoxazole naïve (group 1) and the remaining 39 HIV-infected were on cotrimoxazole prophylaxis (group 2). The prevalence of urinary tract infection (UTI) was 31.5%; 29.5% in group 1 compared to 70.5% in group 2. Cotrimoxazole resistance was recorded in 77% of bacterial strains isolated from group 1 and 84% in group 2 with no statistical difference. In both groups, the most common bacteria isolated were *Escherichia. coli* (*E; coli*) and *Klebsiella. Pneumoniae* (*K. pneumoniae*). These strains were resistant to more than 70% of the antibiotics tested, nevertheless they showed a good sensitivity to ciprofloxacin, imipenem and ceftriazone.

Conclusion: This study shows that despite the use of cotrimoxazole as prophylactic, UTI still occurred in HIV infected children, with a global prevalence of almost four-fifth resistance to cotrimoxazole and almost three-quater multi-resistance to other antibiotics. This implies a requirement of antibiogram in case of UTI before prescription.

Keywords: HIV-exposed Children; Cotrimoxazole; Lower Urinary Tract Infection; Bacterial Resistance

Abbreviations

E. coli : *Escherichia coli;* HIV: Human Immune Virus; *K. pneumoniae*: *Klebsiella pneumoniae;* UTI: Urinary Tract Infections

Introduction

The HIV virus, which causes AIDS, was discovered in 1983. It belongs to the retrovirus family. According to WHO and UNAIDS estimates, 36.7 million people were living with HIV at the end of 2016, its prevalence is 12.6% in South Africa. In Cameroon 3.4% of the population is living with HIV/Aids [1]. To date, 3 modes of transmission have been identified; namely sexual (80%), blood (5 to 10%) and mother-to-child transmission (15 to 45% in the absence of interventions). The World Health Organization (WHO) has established a scheme for monitoring children born to HIV-positive mothers. In addition to antiretroviral treatment (ART), there is prophylaxis with isoniazid (INH), and cotrimoxazole. Cotrimoxazole is a broad-spectrum antibiotic, belonging to the sulfonamide family and composed of sulfamethoxazole and trimethoprim. It is effective against several infections such as bacterial urinary tract infections, broncho-pulmonary infections, tuberculosis, and candidiasis just to name a few [2]. Since their discovery, antibiotics have proven to be very effective in the fight against bacterial-caused diseases affecting humans and animals. Exerting pressure on microorganisms, the overuse of antibiotics is the main epidemiological factor responsible for the emergence of resistance. Many bacterial species have developed resistance mechanisms to several classes of antibiotics. In the hospital setting, the treatment of infections caused by multi-resistant bacteria is becoming increasingly problematic [3]. The existence of multi-resistant bacteria has implications for therapeutics, public health and environmental health. The new WHO Global Antimicrobial Resistance Surveillance System (known as GLASS) reveals that antibiotic resistance is a widespread problem affecting 500,000 people with suspected bacterial infections in 22 countries [4]. This antibiotic resistance creates treatment failures that lead to increased medical expenses, prolonged hospitalizations, and increased mortality, 13,000 deaths per year in France [5]. In the era of HIV/Aids, the use of cotrimoxazole has been increased, as recommended for all PLHIV [5], thus increasing the chance of development of resistance. The objective of this study was to assess the cotrimoxazole resistance profile of bacterial strains isolated from urine samples of children born to HIV-positive mothers.

Methods

A qualitative study with descriptive aim was conducted over a period of 6 months (from 06 May to 29 November 2019). This study was carried out in a pediatric hospital of Yaounde, Cameroon. The sampling technique was non-probabilistic, with a convenience size. The biological fluid analyzed was urine. Was included in this study any child less than 15 years but not less than 2 months old, presenting clinical signs of a urinary tract infection, born to an HIV-positive mother on or not on cotrimoxazole. They were divided in two groups: group 1 constituted of children not infected by HIV and therefore not under cotrimoxazole prophylaxis and group 2 constituted of HIV-positive children under cotrimoxazole.

Ethical considerations

This study was carried with ethical clearance issued by the ethical committee under the number: N° 2018/09/1099/CE/CNERSH/SP. This study was also authorized by the Director of HGOPY, and was carried out according to good practice of medical analysis.

Enrolment, sample collection and biological analyses

Patients in the pediatric consultation unit, presenting signs of a urinary tract infection and who met the inclusion criteria of our study mentioned above were recruited. After been informed of the study objectives, the assent and parental forms were signed. Sampling conditions were explained to the parent and the patient. The sample collected was then taken to the laboratory as soon as possible for analyses.

A macroscopic examination of urine was done to note the color and appearance of the urine. This step was followed by the culture of the urine on Cystine Lactose Electrolyte Deficient (CLED) and Mac Conkey agar with violet crystal. The biochemical analysis of the urine was performed using a urine strip according to the manufacturer instructions (Medi-Test combi 10^{R} SGL) Parameters such as pH, glucose, ketones, leukocytes, nitrites, protein, blood, urobilinogen, and bilirubin in the urine were recorded.

Inoculation on agar

The calibrated loop technique for culture was used. This technique consisted of dipping the calibrated loop vertically into homogenized urine, then making a streak on one radius of the dish to unload the loop and then, without reloading the loop, making

tight and then loose perpendicular streaks over the entire surface of the dish, as shown in figure 1 [7]. The culture was incubated for 18 to 24 hours in an incubator at 37°C.

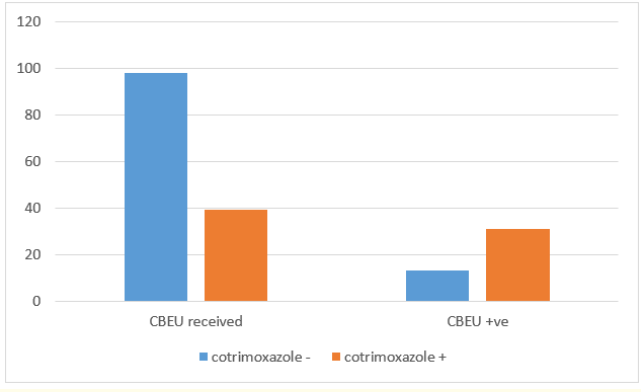

Figure 1: Distribution of the study population according to the result of the CBEU.

The Malassez cell was used to count cells such as red blood cells, leukocytes present in the collected urines. table 1 shows the significance threshold of these cells in case of urinary infection [8,9].

Leucocyturia	> 10/mm^3 or > 10^4/mL
Hematuria	> 10/mm^3 or > 10^4/mL
Bacteriuria	≥ 10^5 UFC/mL

Table 1: Significance thresholds for cell count.

A microscopic observation with X40 objective was performed to identify elements such as epithelial cells, red blood cells, leukocytes, parasite eggs, crystals, cylinders, yeasts. A urine smear was then performed and stained with Gram stain.

As urine is normally sterile, polymicrobia was considered as a contamination. For this purpose, a re-sampling was recommended. A Gram control was done afterwards, using CLED agar.

According to the microscopic observation after Gram stain, in case of Gram-bacilli, an oxidase test was performed, and the incriminated germ identified with the API 20E gallery.

In case of Gram + cocci, a catalase test was performed. This allows to differentiate staphylococci (catalase +) from streptococci (catalase -). In case of positive reactions to catalase, the identification of the germ was performed at the species level for Staphylococcus. For this, 3 tests were done: Mannitol fermentation, coagulase test and DNAse test.

On Mueller Hinton agar, an antibiogram was performed using the antibiotics presented in table 2 below according to the "Comité de l'Antibiogramme de la Societé Française de Microbiologie (CA-SFM) recommendations of 2018.

Antibiotics	Critical diameter established by manufacturer (mm)	Concentration in µg
Enterobacteriaceae		
Amikacin	16 - 21	30
Amoxicillin	16 - 21	25
Nalidixic acid	>20	30
Ciprofloxacin	22 - 25	5
Levofloxacin	17 - 20	5
Gentamicin	16 - 18	15
Imipenem	17 - 24	10
Tetracyclin	17 - 19	30
Tobramycin	16 - 18	10
Cefotaxime	23 - 28	30
Ceftriaxone	23 - 26	30
Aztreonam	21 - 27	30
Cotrimoxazole	13 - 15	25
Staphylococcus only		
Erythromycin	19 - 22	15
Gentamicin	>20	120
Lincomycin	17 - 21	15
Rifampicin	24 - 29	5
Vancomycin	>17	30
Kanamycin	15 - 17	30

Table 2: Antibiotics tested and their critical diameters (CA-SFM 2018).

The diameters formed around the antibiotic disc were measured with a ruler. This zone is called inhibition diameter. These diameters were plotted against the experimentally established critical diameters. The tested strains were considered resistant when the diameters were lower than the first bound of the critical diameter and sensitive when these diameters were higher than the second bound of this diameter. This can be seen in table 2.

Results

Socio-demographic description of the study population

Our study population consisted of 137 children, of whom 78 were female and 59 male with a sex ratio of 1.32 in favor of the female. The most represented age group was 2-5 years old as represented in table 3 of appendix.

Age group	7 months to 2 years	2 to 5 years	6 to 10 years	11 to 15 years
Number of participants	19	56	24	38

Table 3: Age distribution of the population study.

Our study population was divided into 2 groups: group 1, children not on cotrimoxazole prophylaxis (98) and group 2, children living with HIV/Aids on cotrimoxazole prophylaxis (39) (see table 4 of appendix).

HIV - children	HIV + children	Total
cotrimoxazole -	cotrimoxazole +	
98	39	137

Table 4: Distribution of the study population according to HIV status and cotrimoxazole use.

Profile of isolated bacteria

From the 137 urine samples received, 44 (32.1%) were positive for the presence of any bacteria, 28 were from female children and 16 were from male children. Of these 44 positive urine samples, 31 were from children on cotrimoxazole (70.5%) and 13 (29.5%) from cotrimoxazole-naive children. These results are illustrated in figure 1.

Bacterial species isolated

Of the 44 positive urine samples, 4 bacterial species were isolated: 25 *Escherichia coli* (57%), 11 *Klebsiella pneumonieae* (25%), 6 Staphylococci (14%) and 2 *Serratia marcescens* (4%), distributed in the two study groups as shown in figure 2.

Antibiotic resistance profile of isolated bacteria

Most of the bacterial strains isolated from our study population (*Escherichia coli* and *Klebsiella pneumoniae*) were resistant to cotrimoxazole (82%). Of these 82% of bacterial strains, 72% were from HIV+ children (26/36) and the remaining 28% of strains were from HIV- children (10/36). However, these strains were more than 70% sensitive to imipenem, ceftriazone and ciprofloxacin.

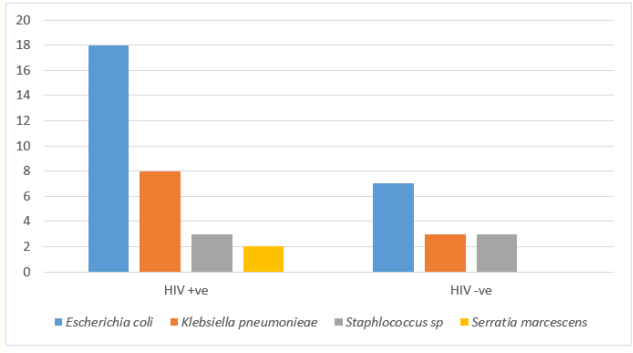

Figure 2: Distribution of bacterial species isolated according to HIV status.

Cotrimoxazole prophylaxis as a risk factor for bacterial resistance

The relative risk to develop resistance to cotrimoxazole was the same for children on cotrimoxazole and those naïve (RR 1.09, P = 0.161) at 95% confidence.

Discussion

Cotrimoxazole use as prophylactic to reduce opportunistic infections in HIV-positive patients greatly reduce morbidity and mortality [10,11]. Since 2006, UNAIDS has recommended that cotrimoxazole be routinely administered to all children exposed to HIV/AIDS, pregnant women, and HIV-positive infants and children, as well as adults. Cameroon has adopted this recommendation since the past 15 years [12]. However, the selective pressure exerted on bacteria due to constant exposure to antibiotics has been noted as the main factor promoting antibacterial resistance development.

The objective of this work was to determine the cotrimoxazole resistance profile of bacterial strains isolated from urine samples of children born to HIV positive. The prevalence of urinary tract infections found in this study was 32.1%. Despite the use of cotrimoxazole to prevent infections, 70.5% of these UTI were found in children taking Cotrimoxazole. The prevalence of UTI found in our study was higher than that found in Yaounde-Cameroon by Kenkouo in the 2008 (15.3%) [8]. This difference could be explained by the difference in sample size of each study or the increase in the prevalence of these infections. On the other

hand, Kouemo and collaborators in their study to determine the bacteriological profile of uropathogenic germs in children under 5 years old in Douala found a prevalence of 32.5%, identical to ours [13]. Of these urinary tract infections, 28 cases (or 64%) were from female subjects. This result shows that urinary tract infections are more recurrent in female than male. This result corroborates with that of Chang and colleagues [14]. The recurrence of UTIs in female subjects could be explained anatomically, the relatively shorter length of the female urethra and the heavy and regular colonization of the perineum by enteric organisms could also explain this result. The age range of 2 to 5 years was the most represented among children with UTI in our study population. This result is similar to that found in the 2017 study by Korbel [15]. This could be explained by the fact that the children in this age group are in the toilet training age.

The bacterial profile found in our study was similar to that of the studies done by Lee in 2018, Dossim in 2017 and Mebarkia in 2016. These studies revealed that *Escherichia coli* was the most incriminated germ with 80%, 42.3% and 45.7% prevalence respectively [16,17]. This could be explained by the fact that *Escherichia coli* is a unique species among bacteria with wide pathogenic behavior. It is ubiquitous and is the predominant species of the facultative aerobic-anaerobic flora of the digestive tract. It possesses adhesion factors that facilitate its adherence to the host epithelium. Once in the uroepithelium, it forms a biofilm to escape attack by the host immune system [18]. The majority of bacterial species isolated in our study showed high resistance to antibiotics commonly used in the treatment of UTIs. This could be explained by the important growth of antibiotic resistance in human medicine.

Overall, 82% resistance to cotrimoxazole was recorded, with 72% (26 of 36) in children on prophylaxis. Powis and collaborators in 2017 found 84.2% resistance among HIV-exposed children [19]. This high resistance could be explained by the relatively low cost of this antibiotic and its generalized use in all socio-economical classes. In addition, it is an over-the-counter drug as such it is a self-prescribed (auto medication). These criteria could contribute to the abuse of this antibiotic, and consequently a high frequency in the development of resistance. All strains of *Escherichia coli* and *Klebsiella pneumoniae* isolated in our study showed total resistance to cotrimoxazole. This was highlighted in another study undertaken at Bugando Medical Center by Karol., *et al*. in 2015, who found that these species are the most common bacteria among Gram-negative bacteria developing cotrimoxazole resistance [20]. Moroh., *et al.* in 2013 demonstrated 77.9% resistance of *E. coli* to cotrimoxazole in their studies investigating bacterial diversity and antibiotic resistance in urinary tract infections in Côte d'Ivoire [21]. The percentage of cotrimoxazole resistance was high in both groups (77% in the HIV-negative group and 84% in the HIV-positive group). This is consistent with the study by Morpeth and collaborators on the effect of cotrimoxazole prophylaxis and *E. coli* antimicrobial resistance in HIV patients in Tanzania in 2008 [22]. Of the antibiotics tested, we noted a better susceptibility profile to ceftriaxone, imipenem, and ciprofloxacin. This corroborates with Beneme's 2014 study showing that ciprofloxacin and ceftriaxone have better sensitivities to UTI causative bacteria [23]. Similarly, recent studies conducted in Greece and Iran, respectively, with the objective of determining antibiotic resistance of UTI causative germs in children showed good antimicrobial activity of third generation cephalosporins (98.3%), ciprofloxacin (98.6%) and imipenem (90.1%) [24,25]. Of these strains, *E. coli* and *K. pneumoniae* showed a mean resistant profile to amoxicillin and gentamicin. No matter the decrease in the consumption of cotrimoxazole in countries like in the UK, authors have questioned if this antibioresistance will ever go down [26].

Although we enrolled few children, the results obtained present to us the persistence and evolution of the resistance to cotrimoxazole. Our results further fueled the question about stopping or continuous usage of cotrimoxazole in HIV patients.

As well, this study could have a limitation because antibiotic resistance was determined by the agar diffusion method instead of the reference method in liquid medium, but results obtained are still indicative of the resistance profiling.

Conclusion

Our results show that UTI was more prevalent in HIV infected children on cotrimoxazole; but the prevalence of resistance to cotrimoxazole was like that in children naïve of cotrimoxazole. Isolated strains presented mutiresistance profile, but were still sensitive to other antibiotics. This implies that antibiogram should be done in case of UTI before prescription. Equally there was no statistically significant difference in the antibioresistance between the two groups. These findings are reassuring, given current recommendations for long-term cotrimoxazole prophylaxis

among children living with HIV in Africa to decrease mortality and morbidity. Nervertheless, it will be advisable to multiply studies on this domain so as to review WHO recommendation on the use of cotrimoxazole as prophylaxis by PLHIV.

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