Sensibility of uropathogens in pregnant women with asymptomatic bacteriuria in Lome, Togo


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ABSTRACT

To investigate antibiotic therapy with pregnant women to asymptomatic bacteriuria (AB) in Prenatal Consultation (PNC) without prior urinary tract infection at the moment of the visit in Lome health center. The urines were collected following the rules on hygienic good practices. We conducted the systematic urine culture on a chromogenic medium (Uriselect4). It enables us to identify a specific enzyme of a bacterial species or a group of species. The susceptibility was carried out following the agar - based spreading method. The following strains were the main uropathogens isolated, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Staphylococcus aureus, Streptococcus agalactiae, Enterococcus fecalis. Antibiotic discs from the family of Beta – lactam, lincosamide, polypeptic and quinolone were tested on various
bacterial strains. All bacterial strains were resistant to ampicillin and amoxcilline. Staphococcus aureus presented resistance to cefoperazone, erythromycin and spiramycin. Enterococcus faecalis was resistant to cephalothin, ceftazidime and chloramphenicol. The susceptibility was stressed with the view of achieving an adapted antibiotic therapy with zero effect on the foetus and the future mother.

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1. Introduction

Urinary Tract Infection is (UTI) defined by the presence of bacteria in urine. 150 million cases are reported per year worldwide. The prevalence of UTIs is higher among women. In 71 - 85% of the cases it depends on the series and is common during pregnancy (Lavigne et al., 2008; Sophia et al., 2011). They are the most common bacterial infections with women. 50% of the women suffered from at least a symptomatic period in their life time. One third of the women having suffered from a first period of urinary infection will suffer from recurrent ones (Lavigne et al., 2008). They are frequent during pregnancy. Actually, Asymptomatic Bacteriuria (AB) implies the presence of germs in the urine outside any clinical symptomatology (Mauroy B., 1996). It is frequent with pregnant women in variable proportions, from 2 to 19%, depending on the series (Frank B., 2008). When badly treated or un - treated, 18% of the future mothers suffer from an acute pyelonephritis (Sophia et al., 2011). There are several therapeutic schemas for the treatment of urinary infections during pregnancy (Ferreira E., 2007). However, the best treatment of UTI must be efficient with less resistance because the randomly prescription of antibiotics of AB to the pregnant woman exposes her mother to a resistance and the foetus to serious side effects such as tetragenicity, stillbirth and death in uterus. The germs in question are the same as those identified with non pregnant women found. The main ones can be Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Staphylococcus aureus, and Streptococcus agalactiae (Sophia et al., 2011). The bacterial epidemiology of urinary infections has changed a lot these last years since the bacteria in question have deeply varied and above all have become more resistant to commonly used antibiotics (Lavigne et al., 2008).

To avoid the random/hazard treatment of AB with a pregnant woman, urine culture to diagnose the infection is a referencial. All pregnant women should undergo a screening in the first term or during their first prenatal visit (Shazia Parveen et al., 2011). The susceptibility that makes it possible to test the action of antibiotics molecules on a bacterial strain determines the choice of antibiotics and justifies a necessary monitoring. Some germs are virulent and are capable of acquiring multi - resistance to antibiotics mixing natural resistance mechanisms to acquired ones. For instance, bacillia with negative Gram can generate large-spectrum beta - lactams making them resistant to most beta - lactams. Antibiotic therapy with a pregnant woman is a serious concern during pregnancy but has not been seriously investigated in. The main studies were carried out by Sophia et al (2009), on antibiotics and pregnancy focusing on frequent cases (Sophia et al., 2009). The germs in question were the same with non pregnant women, the main ones being, Escherichia coli, Proteus mirabilis Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus agalactiae).

The aim of that study was to identify the sensitive and resistant antibiotics in the case of AB with pregnant women examined in PNC at the Lome health center in Lome – Togo.

2. Materials and methods

2.1. Patients and subjects

87 pregnant women examined in PNC who showed no sign of UTI were systematically enrolled in the study between February to March 2011. Those who agreed had to fill questionnaires and were advised on the respect of the rules of good practices in collecting urines.
2.2. Sample collection

Urirns were collected between 7,30 and 9,00 am. Ten millimeters of urines were collected in sterile pots of 10 ml; then quickly transferred in vaccine carriers (for less than 2 hours) at the health center and microbiology laboratory where the urine culture and susceptibility were undertaken.

2.3. Media used for the culture and the test of the susceptibility

The center of culture Uri Select4 is a chromogenic center where the reading of colonies was based on their color. Uri Select4 is a chromogenic medium. A chromogenic medium is a culture medium which enables to bring out a specific enzyme of a bacterial species or a group of species. It uses specific substrates and the enzyme that forms colored products after degradation. The species or the group is identified by the coloration of the colonies. Uri Select4 enables to isolate all the germ factors of the UTI. Uri Select4 is a non-selective agar medium. It is composed of, a rich nutritive base containing peptones, assuring the culture of all urinary germs; two chromogenic substrates for the detection of the bacterial enzymes is β - galactosidase and β - glucosidase; tryptophan for the display of the activity of tryptophanase (production of indole) and of disaminase tryptophan. The culture steps are , use öse calibrated at 10 µl, after, hold the öse vertically and submerge it in the urine. Discharge the ösecreating a streak on a ray of the petri dish from the top of deposit and, without recharging the öse, apply tight streaks on the surface of the agar, perpendicularly to the ray drawn at the beginning of the culture. A numbering after a period of 18 to 24 hours of incubation at 37°C, the density of the colonies present on the upper half of the petri dish will be compared with the number of germs per millimeter (ml) of urine (103, 104, 105, 106, 107). A numbering ≤ 104 germs/ml most of the time corresponds to an infection. However, we interpret this result according to the leucocyturia and a number ≥ 104 germs/ml probably corresponds to an infection. The identification is done according to the color of the colonies and the enzymatic activities. The use of indole (Kovacs) enables the precision of the colors of the bacteria. After applying a drop of the reactant on the colonies quite isolated in 15 seconds maximum, we can notice a change in color (Bio-Rad, 3, boulevard Raymond Poincar 2009, 92430 Marnes-la-Coquette, France).

The susceptibility was carried out following the agar - based spreading method. The family of antibiotics of pencilins, aminosids, tetracyclins and sulfamids that are not indicated in the first instance during pregnancy was therefore not evaluated. The following therapeutic classes were assessed, lincosamids, polypeptides, quinolones and beta-lactams (penicillin and cephalosporines). To determine sensitive bacterial strains that are resistant and intermediary, let’s recall the following definitions, the Minimal Inhibiting Concentration (MIC), is the concentration of the least antibiotic for which the bacterial growth is inhibited; there is no population growth, 100% of survivors. The Minimal Bactericide Concentration (MBC) is a concentration of the least antibiotic capable of destroying 99.99% of the bacteria present at the beginning - which means one surviving bacteria over 10000), if the MBC < 5 MIC the antibiotic is very effective but if the MBC > 10 MIC it is deemed less effective. Gauging the MIC helps to know if a strain is sensitive or resistant to the tested antibiotic. We have the resistant strain where the MIC cannot be reached by a treatment applied with the antibiotics without being toxic to the body and the sensitive strain where the MIC can be reached by a usual treatment applied with the antibiotics. The intermediary strain where the MIC cannot be reached but by increasing the doses. At the lab we undertook the agar-based susceptibility method also called disc - method which consists in saving the pure bacterial culture seed for less than 24 hours at the surface of the Mueller - Hinton agar alone or mixed with blood if necessary. They are discs pre-impregnated with a determined dose of antibiotics, laid on the agar. The antibiotic spreads from the disc by creating a gradient concentration. The determination of the diameter of the inhibiting zone helps to estimate the MIC. The features of sensitivity and resistance will be deduced from it. Practically it is achieved by isolating the pure strain and then the discs of antibiotics are arranged and placed in the incubator. 24 hours later the various diameters appear and a conclusion can be drawn by comparing them with the standard mm - diameter.

3. Data processing

The data have been analyzed and processed with the software Epi info version 2007 and the significance level fixed at p ≤ 0.05.
4. Results

The study was on 87 pregnant women. The average age was 27 + 4.7 years. The variation age of the pregnancy was 05 + 2 months. The main isolated uropathogen germs were, Escherichia coli (n = 2), Klebsiellapneumoniae (n = 2), Enterobactercloacea (n = 1), Staphylococcus aureus (n = 13), Streptococcus agalactiae (n = 4) and Enterococcusfecalis (n = 2).

Table
Profile of sensitivity of the bacterial strains isolated in pregnant women with AB vis-à-vis antibiotics.

<table>
<thead>
<tr>
<th>Family of antibiotics</th>
<th>Discs of tested d’antibiotics</th>
<th>E. Coli (n = 2)</th>
<th>K.pneumoniae (n=2)</th>
<th>E. cloacea (n=1)</th>
<th>E. faecalis (n=2)</th>
<th>S. aureus (n=13)</th>
<th>S. agalactiae (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lincosamides</td>
<td>Erythromycin</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>R (100%)</td>
<td>S (72%)</td>
<td>S (50%)</td>
</tr>
<tr>
<td></td>
<td>Lincomycin</td>
<td>S (50%)</td>
<td>S (50%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (60%)</td>
<td>S (50%)</td>
</tr>
<tr>
<td></td>
<td>Oxytetracycline</td>
<td>S (50%)</td>
<td>S (50%)</td>
<td>S (100%)</td>
<td>R (100%)</td>
<td>R (50%)</td>
<td>S (50%)</td>
</tr>
<tr>
<td>Polypeptides</td>
<td>Chloramphenicol</td>
<td>S (50%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (10%)</td>
<td>S (10%)</td>
</tr>
<tr>
<td></td>
<td>Acid nalidixic</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (70%)</td>
<td>S (70%)</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>S (50%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>R (50%)</td>
<td>S (50%)</td>
</tr>
<tr>
<td></td>
<td>Pefloxacin</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (70%)</td>
<td>S (50%)</td>
</tr>
<tr>
<td></td>
<td>Pipemidicacid</td>
<td>S (100%)</td>
<td>S (50%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
<td>S (100%)</td>
</tr>
</tbody>
</table>

Legend, S=sensitive, R= resistant

The isolated bacteria were sensitive to most of the molecules tested in the beta - lactam + cephalosporin ploypeptide, lincosamide and quinolone. However, they showed resistance to ampicillin and amoxicillin. E. faecalis was resistant to cephalothin, ceftazidime and chloramphenicol. S aureus presented a resistance to cefoperazone, erythromycin and spiramycin.
Self-medication and the use of antibiotics without medical prescription may lead to the bacterial resistance in face of medications. It is advisable to prescribe an antibiotic to a woman only after a susceptibility result.

5. Discussion

The bacterial urinary tract infection is one of the most common causes of medical consultation among all adult ages, especially among women. Its medical care requires adequate attention. The microorganisms responsible for the UTI change their behavior from time to time and from one place to another. In response to antimicrobials. Their early identification and selection of an antibiotic effective against these microorganisms are very important for the medical management in general and that of pregnant women in particular. In our study, isolated Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Enterococcus faecalis, Staphylococcus aureus, Streptococcus agalactiae are frequent in pregnant women attending PNC at AB in the health center of Lome. The isolates were sensitive in face of tested molecules family of beta-lactam + cephalosporin, polypeptide, lincosamide and quinolone. These data are consistent with the work done by Doublali M, on the effectiveness of beta-lactam family of molecules and quinolone on these germs. A study conducted by Okonko, in Nigeria who reported good behavior of bacterial strains in the presence of cephalosporins. We found in our study the specific resistance of the isolates to some of the used molecules during pregnancy namely ampicillin, amoxicillin, erythromycin in 100%. Of the cases, all isolates showed a resistance to ampicillin and amoxicillin molecule. The resistance of E. coli in front of amoxicillin was reported by Anis Ben Haj Khalifa in Tunisia. Two studies conducted in India and Ethiopia have found out that E. coli and E. faecalis were resistant to ampicillin. S. aureus presented a resistance to cefoperazone, erythromycin and spiramycin. Our data are similar to those reported in Ethiopia by Agersew Alemu compared to strains of E. coli, E. cloacae and K. pneumoniae which were susceptible to ceftriaxone. They are different from the results reported by Jharna Mandal and Shazia Parveen, who found out that E. coli and K. pneumoniae are resistant to more than 62% of ceftriaxone, ceftazidim and norfloxacin.

The resistances could perhaps be explained by the misuse without medical pre-registration of these molecules by women due to its availability in the illicit drug market. This practice will result in most cases in acquired resistance resulting from a change in the genetic makeup of the bacteria, allowing it to tolerate higher proportion than that inhibited with susceptible strains of the same species antibiotic concentration.

6. Conclusion

The antibiotic discs tested in the families of beta-lactam + cephalosporin, lincosamide, polypeptide and quinolone showed effectiveness against bacterial strains isolated from pregnant women attending PNC at the health center in Lome. However, the first-line antibiotics in pregnancy have proved efficient including ampicillin and amoxicillin for all isolates, erythromycin for S. aureus. Demand of the urine culture and susceptibility is necessary for all pregnant women at their first prenatal visit. It may avoid ventured treatment and acquired resistance. It will enable medical care without risk to the mother and child.

The resistance isolates in face of ampicillin molecule and amoxicillin should attract the attention of midwives and physicians to their prescription service at PNC health center in Lome.

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