

Profile and antimicrobial resistance to newly available drugs of urinary tract pathogens among Malagasy pregnant women

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Abstract. Urinary tract infections are caused by growth of microorganisms anywhere in the urinary tract and pregnant women are particularly susceptible to it. Collection of baseline information regarding urinary tract bacterial profile and their antimicrobial susceptibility in pregnant women are important for treatment purposes. In order to determine the distribution of community-acquired urinary tract pathogens and estimate their susceptibility patterns to antimicrobial agents currently available in Antananarivo (Madagascar), we conducted a cross sectional preliminary survey (four months) on bacteria isolated from urine of pregnant women attending antenatal care clinic, Ambohimandra Hospital Center. Fifty pathogens were isolated of which 21 (42%) were Gram-negative bacilli and 29 (58%) Gram-positive cocci. The most commonly isolated pathogen was Coagulase Negative Staphylococci (13 strains) for Gram-positive cocci and *Escherichia coli* (6 strains) for Gram-negative bacilli. Thirty three percent of Gram-negative bacilli were resistant to 3GC. One *Klebsiella pneumoniae* strain was found to be resistant to imipenem and one Group B *Streptococci* strain was vancomycin resistant. This development of resistance to newly available antimicrobial and the increase proportion of Gram-positive cocci strains imply that a drastic antimicrobial resistance surveillance need to be carried out to ensure appropriate empirical treatment.

INTRODUCTION

Urinary tract infections (UTIs) are caused by growth of microorganisms anywhere in the urinary tract. Women are particularly susceptible to UTI, mainly due to short urethra, absence of prostatic secretion and easy contamination of the urinary tract with fecal flora due to the proximity of the anus (Haider *et al.*, 2010). Pregnancy is considered as factors that increases the risk of UTI partly due to (i) the pressure of gravid uterus causing stasis of urine flow (ii) humoral and immunological changes that occur during normal pregnancy (Ramzan *et al.*, 2004). Such infection could lead to adverse pregnancy outcome such as pre-mature labor. UTI can be reduced through health

education, good personal hygiene, repeated urine cultures early in pregnancy and during the third trimesters, and to treat UTI with specific antimicrobial agent. In most developing country such as Madagascar, lack of biological investigation leads to empirical treatment that requires information regarding urinary tract bacterial profile and their susceptibility to antimicrobials.

In Malagasy community, urinary tract pathogens are usually dominated by Gram-negative bacilli, and particularly *Escherichia coli* (Randrianirina *et al.*, 2007). Randrianirina *et al.* reported that Gram-positive bacteria (*Streptococcaceae* and *Staphylococcus* spp.) were responsible for 10% community-acquired urinary tract infections. According to two Malagasy

studies, urinary tract pathogens are becoming increasingly resistant to commonly used antibiotics particularly those that are available at a low prices such as amoxicillin and trimethoprim/sulfamethoxazole (Randrianirina *et al.*, 2007; Rasamiravaka *et al.*, 2015). Since the last decade increased usage of cephalosporin and particularly third-generation cephalosporin for empirical treatment of UTIs in Madagascar has been noticed. Glycopeptide (such as vancomycin) and carbapenem (such as imipenem) are considered as last resort treatment against MRSA and BLSE multidrug resistant bacteria that are recently available in Malagasy hospitals. There is high risk for development of resistant strains to third-generation cephalosporin, carbapenem and vancomycin. As no relevant study have been reported elsewhere, this, preliminary study has been carried out to provide baseline line information regarding antimicrobial resistance patterns in urinary tract bacteria among pregnant women to β -lactamine (particularly 3GC and carbapenem) and glycopeptide; such data could be helpful for empirical therapy.

MATERIALS AND METHODS

Study and samples recruitment

In order to obtain updated data on (i) the distribution of pathogens responsible for community-acquired UTIs in Malagasy community, and (ii) their susceptibility patterns to newly available antimicrobial agents, we carried out a preliminary study on bacteria isolated from the urine of consenting pregnant women attended the antenatal care clinic of Ambohimandra hospital (Antananarivo). Bacterial isolation and identification as well as antimicrobial susceptibility test have been carried out at the Laboratory of Training and Research in Medical Biology. Age, gestational age and parity were mentioned through succinct anonymous questionnaire without any consideration of asymptomatic bacteriurea and symptomatic UTI.

1. Culture and identification

Urine collection following usual recommendation and all specimens were transported from the hospital to regional laboratory and processed within 2 hrs (Moinard, 1987). Those specimens which could not be processed immediately were not kept in refrigerator and processed no longer than 18 hours after collection.

Isolation and identification of pathogens were conduct by using classical culture methods. Briefly, collected urine sample were inoculated Uriselect® culture media and incubated aerobically for 24 hours at 37°C. Then isolated bacteria were identified by biochemical characteristic. The standard reference strains; *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used for quality control of culture media. Specimens which produced $\geq 10^5$ CFU/ml of urine were considered significant and $< 10^5$ CFU/ml were considered insignificant or due to contamination.

2. Antimicrobial susceptibility tests

Susceptibility to antibiotics was assessed according to the recommendations of the Antibiogram Committee of the French Microbiology Society (Skov *et al.*, 2007). For Gram-negative bacilli, antimicrobial agents tested include amoxicillin (AMX), two third-generation cephalosporins (3GC, cefotaxim and ceftazidim) and one carbapenem (imipenem, IMP). Penicillin (PEN), oxacillin (OXA) and vancomycin (VAN) were tested for Gram-positive cocci. Antimicrobial susceptibility of Gram-positive bacilli was not tested if isolated.

3. Data analysis

For statistical analyses, χ^2 at $p < 0.05$ was considered significant for all comparisons with the help of GraphpadPrism5 software (Avenida de la Playa, La Jolla, CA, USA). Risk factors were also evaluated and variables achieving a $P < 0.05$ level were considered significant; odds ratios (ORs) with 95% CIs were calculated by using the Graphpad prism5® software.

RESULTS

Fifty infected pregnant women were selected from 17 to 38 years (with a mean \pm SD age of 26 ± 17.55 years) that represent 34.24% of the pregnant women (n=146) who gave their consent.

UTI were significantly higher among women aged less than 20 years (OR, 5.31, 95% CI, 2.365–11.907; $P < 0.0001$) and lower among the 1st trimester and multiparous pregnant women (OR, 0.02, 95% CI, 0.003–0.214; $P < 0.0001$; OR, 0.11, 95% CI, 0.015–0.937; $P = 0.019$, respectively) (Table 1). Among infected women, no significant correlation was observed between gestational age and bacterial pathogen group. Gram-positive cocci group were more frequent among 2nd trimester pregnant (Table 1). In contrast, cocci group is significantly higher among primiparous (OR, 5.56, 95% CI, 1.622–19.03; $P < 0.009$) while multiparous were mostly infected by Gram-negative bacilli (Table 1).

Fifty pathogens have been isolated with 21 (42%) Gram-negative bacilli and 29 (58%) Gram-positive cocci. Two patients presented coinfection with Gram-negative bacilli and Gram-positive cocci. Among Gram-negative bacilli, the most commonly isolated bacteria were *Escherichia coli* (6 strains) and *Klebsiella pneumoniae* (3 strains). None strains presented sensitive phenotype with respect to their natural resistance. All Gram-

negative bacilli were resistant to amoxicillin and 11 strains were resistance to one or both 3GC tested (Detailed in Table 2). Resistance to imipenem was observed for one *K. pneumoniae* strain (Table 2). Gram-positive cocci pathogens were dominated by the Streptococci group (n=16) and coagulase-negative *Staphylococcus epidermidis* (n=10). Only three *Staphylococcus aureus* have been isolated and all of them were resistant to oxacillin but sensitive to vancomycin. Streptococci isolated were dominated by Group B *Streptococci* (n=9) and *Enterococcus sp.* (n=7). One Group B *Streptococci* has been found to be resistant to vancomycin. Finally, None *Staphylococcus saprophyticus* and uncommon bacteria have been isolated.

DISCUSSION

Infected pregnant women represent 34.24% of all consenting participants. The Malagasy pregnant women had a higher infection rate compared to general population and pregnant women from other communities (Dimetry *et al.*, 2007; Demilie *et al.*, 2012). However, poor genital hygienic practices and availability of water could explain the high rate of UTI in developing countries. The low rate of infection among pregnant women (n=1; $p < 0.003$) in first trimester are understandable as there is shortening of urethra that could

Table 1. Distribution of isolated bacteria group according to parity and gestational age among pregnant women

Isolated bacteria	Number of strains	State of pregnancy								
		Age			Gestational age			Parity		
		<20	20-35	35-50	1 st T*	2 nd T	3 rd T	<2	2 or 3	>3
<i>Gram-positive cocci</i>	29 (19.86)	13	14	2	0	14	15	20	8	1
<i>Gram-negative bacilli</i>	21(14.38)	10	10	1	1	6	14	6	15	0
<i>Negative culture</i>	96 (65.75)	13	70	11	20	32	44	40	42	14
<i>p</i>		<0.0001			<0.0001			0.009		0.019

* Trimester

Table 2. Distribution of isolated bacteria and resistance phenotype among pregnant women

Isolated bacteria	Number of strains (%)	Resistant phenotype					
		PEN	OXA	VAN	CTX	CAZ	IMP
<i>Staphylococcus epidermidis</i>	10 (20)	10	5	1	ND	ND	ND
Group B Streptococci	9(18)	9	9	0	ND	ND	ND
<i>Enterococcus sp.</i>	7(14)	7	7	0	ND	ND	ND
<i>Staphylococcus aureus</i>	3(06)	3	3	0	ND	ND	ND
<i>Escherichia coli</i>	6(12)	ND	ND	ND	1	5	0
<i>Salmonella sp.</i>	5(10)	ND	ND	ND	1	2	0
<i>Klebsiella pneumoniae</i>	3(06)	ND	ND	ND	1	2	1
<i>Enterobacter cloacae</i>	3(06)	ND	ND	ND	1	1	0
<i>Proteus mirabilis</i>	3(06)	ND	ND	ND	0	1	0
<i>Pseudomonas aeruginosa</i>	1(02)	ND	ND	ND	0	0	0

ND: not determined

not be achieved with lightweight fetus. However, they represented a very limited group (n=21) which could limit statistical significance. It is difficult to interpret the lower rate of UTI in multiparous pregnant (n=15). In contrast, high rate of infection was observed in women under 20 years of age (n=23; p<0.0001) and higher rate of infection by cocci in primiparous women (n=20; p=0.009) are intriguing. Other risk factors such as sexual activity which is generally associated with young pregnant women should be addressed to shed light.

When comparing the rate of isolates of Gram-negative and Gram-positive bacteria, Gram-negative bacteria were not the dominant causative agent of UTI compared to other studies (Lewis *et al.*, 2013; Okonko *et al.*, 2009, Sescon *et al.*, 2003; Prakash & Saxena, 2013). This increase rate over fifty percent of Gram-positive cocci responsible of UTI is intriguing. However, other recent studies in India reported more than 20% of Gram-positive cocci to be responsible of UTI (Dash *et al.*, 2013) and similar result have been notified in Nigeria and pointed out the *high prevalence of S. aureus* (32.35%) (Oladeinde *et al.*, 2011; Omoregie *et al.*, 2008). In Ethiopia, similar rate (33%) has been observed among pregnant women (Demilie *et al.*, 2012) although *E. coli* remains the most common detected bacteria.

It's common to isolate numerous common gut microbiota such as *Enterobacteriaceae* and *Enterococcus sp.* However, high rate of Gram-positive cocci particularly compared to Gram-negative bacteria is particularly intriguing. Although previous study conducted in our lab already pointed out the prevalence shift of uropathogens to Gram-positive cocci (Rasamiravaka *et al.*, 2015), we could not proposed reasonable hypothesis. Evaluation of gut flora of pregnant women should be addressed to establish concrete connection between gut microbiota and uropathogens.

Data distribution of uropathogens is largely important for country and locality with poor access to health care services and commonly used empirical antibiotics. Thus, this uncommon prevalence shift of uropathogens to Gram-positive cocci could be problematic for the instauration of adequate treatment of UTI in some area of Malagasy community where bacteriological examination is not systematically realize in order to compensate the sensibility lack of dipstick test which fails to detect non-nitrite producer bacteria such as Gram-positive cocci. Moreover, UTI are usually treated without antibiogram due to cost limitation which could lead to a treatment failure and increase of resistant strain rates. Herein, high rate of resistance to third-generation

cephalosporins in *Enterobacteriaceae* strains as well as high rate of MRSA strains (100 %) put question in the way that 3GC and oxacillin are not anymore suitable for treatment of UTI. A multicenter study on antimicrobial resistance in representative Malagasy community should be lead to determine whether the resistance patterns and the increase in resistance to third-generation cephalosporins are similar. Besides, molecular characterization of isolated resistance strains stills to be carried out.

Some methodological limitation of our study concern (i) lack detection of extended-spectrum beta-lactamase (ESBL) phenotype strains as we do not assess amoxicillin with clavulanic acid (AMC) susceptibility for out of stock reason, (ii) the research of risk factor associated UT bacterial carriage such as antibiotic treatment and hospitalization socioeconomic classes and those with a past history of UTI anatomic urinary tract abnormalities (Turpin *et al.*, 2007; Sheikh *et al.*, 2000), (iii) and we only focus on newly available antibiotic such as vancomycin and imipenem but not on those already available since last decades. Those limitations should be addressed in next investigation.

In conclusion, the prevalence of UTI in pregnant women at Ambohimiandra hospital is comparable with other previous studies in African and other developing countries elsewhere in the world. However, our study findings shown high rate of Gram positive bacteria that is not detectable by urine dipstick test. Moreover, an increase in 3GC resistance rate is noticed. UTI will cause serious problem on both woman as well as on the fetus life in case inadequate care. Although lack of resistance to vancomycin and imipenem are reassuring we invite decision-maker to build polities approach for availability of systematic urine culture and antimicrobial susceptibility test for pregnant women.

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