High prevalence of antibiotic resistance among bacteria isolated from pregnant women with asymptomatic urinary tract infections in Dar es Salaam,Tanzania

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Abstract

Objectives: Urinary tract infections (UTI) are among the major causes of morbidity in pregnant women because of physiological changes during pregnancy and, if unattended to may cause severe complications to mother and foetus. Asymptomatic urinary tract infections (AUTI) can be important predictors for pathogenesis of UTI. This study aimed to determine the prevalence of AUTI among pregnant women using rapid dipstick and routine culture methods to assess antimicrobial resistance patterns of isolated pathogens.

Methods: This was a cross-sectional study. Using non-probability convenience sampling technique, a total of 202 pregnant women attending Antinatal Care (ANC) were recruited. Mid-stream urine was collected and subjected to rapid dipstick and urine culture media. Antibacterial susceptibility tests were conducted against the bacteria. Risk factors for AUTI and demographic data were obtained using pretested questionnaire. Data were analyzed using SPSS Vs. 20 software package.

Results: Of the 202 pregnant women, 47 (23.3%) had AUTI. Age ranged from 15-45 years with a median of 26±3.0 years. The main uropathogens were *Escherichia coli* (*E.coli*) 22(46.8%). The dipstick test had sensitivity of 79.2% and specificity of 63.6%. Seventy-three percent of *E. coli* were resistant to ampicillin and 86% to erythromycin. The prevalence rate of antibiotic resistance among the isolated bacteria ranged from 73 to100%.

Conclusion: Prevalent uropathogens among bacteria isolated from pregnant women with AUTI were *Escherichia coli* and *Klebsiella pneumoniae.* High antibiotic resistance were observed among the bacteria. About 23% of pregnant women had AUTI. We advocate for regular culture of urine to be performed because of the lower sensitivity of dipstick test.

Key-words: Pregnancy, antibiotic resistance, uropathogenic bacteria

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Haute prévalence de la résistance aux antibiotiques chez les bactéries isolées de femmes enceintes atteintes d'infections urinaires asymptomatiques à Dar es-Salaam, en Tanzanie

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Resume

Objectifs: Les infections des voies urinaires (UTI) sont parmi les principales causes de morbidité chez les femmes enceintes à cause des changements physiologiques pendant la grossesse et, si elle n'est pas surveillée, peut causer de graves complications à la mère et au foetus. Les infections asymptomatiques des voies urinaires (AUTI) peuvent être des prédicteurs importants pour la pathogenèse de l'UTI. Cette étude visait à déterminer la prévalence de l'AUTI chez les femmes enceintes à l'aide d'échantillons rapides et de méthodes de culture de routine pour évaluer les caractéristiques de résistance aux antimicrobiens des pathogènes isolés.

Méthodes: il s'agissait d'une étude transversale. En utilisant une technique d'échantillonnage sans probabilité, un total de 202 femmes enceintes participant à l'ANC ont été recrutés. L'urine à mi-parcours a été recueillie et soumise à une jauge rapide et à des milieux de culture d'urine. Des tests de sensibilité antibactériens ont été effectués contre la bactérie. Les facteurs de risque pour l'AUTI et les données démographiques ont été obtenus en utilisant un questionnaire prétesté. Les données ont été analysées à l'aide du logiciel SPSS Vs20.0.

Résultats: Parmi les 202 femmes enceintes, 47 (23,3%) avaient une AUTI. L'âge variait de 15 à 45 ans avec une médiane de 26 ± 3,0 ans. Les principaux uropathogènes étaient Escherichia coli (E. coli) 22 (46,8%). Le test de la jauge a eu une sensibilité de 79,2% et une spécificité de 63,6%. Soixante-treize pour cent de E. coli étaient résistants à l'ampicilline et 86% à l'érythromycine. Le taux de prévalence de la résistance aux antibiotiques chez les bactéries isolées variait de 73 à 100%.

Conclusion: Les uropathogènes prévalents parmi les bactéries isolées de femmes enceintes avec AUTI étaient Escherichia coli et Klebsiella pneumoniae. Une forte résistance aux antibiotiques a été observée chez les bactéries. Environ 23% des femmes enceintes avaient une OAU. Nous préconisons une culture régulière de l'urine à effectuer en raison de la sensibilité plus faible du test de la jauge.

Mots-clés: Grossesse, résistance aux antibiotiques, bactéries uropathogènes

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INTRODUCTION

Over 10% of hospital visits by women are due to urinary tract infections (UTI) particularly during pregnancy (1), which can manifest as asymptomatic urinary tract infections (AUTI) or symptomatic UTI (2). Pregnant women are at increased risk of acquiring UTI with increase in gestation period, from 0.8% in the 12th week to 2% at the end of pregnancy, which could be due to ureteral dilatation (3-5). Other most frequent physiological changes during pregnancy include increased bladder volume and reduced bladder tone accompanied with ureteral muscle tone relaxation. These contribute to increased urinary stasis and ureterovesical reflux (5,6). Besides, majority of pregnant women develop glycosuria that also encourages bacterial growth in the urine (7,8).

Escherichia coli, *Klebsiella* and Enterobacter are found to be among the most frequent causes of UTI, though other gram positive bacteria have been reported (9). Ascending UTI, premature birth and foetal mortality are the frequent complications owing to UTI during pregnancy (10-12). Similarly, one recent study has associated UTI during pregnancy and early occurrence of autonomic nervous system disorders in new-borns (13).

Over the past 5 years, no study had been conducted in Dar Es Salaam to investigate the prevalence rate of UTI during pregnancy and its associated complications. Therefore, there are insufficient local data on AUTI among pregnant women in Dar Es Salaam. Moreover, data on prevalence rates of UTI in Tanzania vary significantly from one administrative or geographical area to another ranging from 14% to 21% (9,14). Amoxicillin constitutes the first line antibiotic therapy for treatment of UTI during pregnancy as per the Tanzania Standard Treatment Guidelines (STG). The second line therapy comprises nitrofurantoin and amoxicillin/clavulanic acid. In case of penicillin sensitive women; erythromycin or azithromycin are the drugs of choice (15). On the other hand, antibacterial susceptibility patterns on newly introduced antibacterial agents such as erythromycin and azithromycin are not well documented. Therefore, data on the prevalence of AUTI during pregnancy and antimicrobial resistance patterns of the pathogens in Tanzania is inadequate. Moreover, early detection and treatment of UTI were proposed in the millennium development goals for health as one of the most cost-effective strategies to reduce incidences of UTI and the associated complications in pregnant women and neonates, particularly in developing countries (16-18). Consequently, this study aimed to identify the current prevalence rates of AUTI among pregnant women attending antenatal clinics (ANC) in Dar Es Salaam and antimicrobial resistance patterns of the isolated uropathogenic bacteria. The acronym AUTI will be used interchangeably with significant presence of bacteria in urine (significant bacteriuria), throughout this paper.

MATERIALS AND METHODS

Study setting/population

This study involved pregnant women attending ANC in Dar Es Salaam City, which is the country's most populous city with over 5 million habitants. The study was conducted at two public health centers viz. Mnazi Mmoja and Magomeni situated in Ilala and Kinondoni Municipalities respectively. The participants include pregnant women attending ANC. The exclusion criteria include symptomatic pregnant women with febrile symptoms including dysuria, urinary hesitancy, incontinence, incomplete voiding and individuals on antibiotic therapy during the study period.

Study design and sampling

A descriptive cross-sectional study was conducted at the two health centers between January and March 2015. The non-probability convenience sampling technique was employed to recruit a total of 202 pregnant women by using the formula:

$$n = \frac{Z^2 p(1-p)}{e^2}$$

Where: z = Z score for 95% confidence interval = 1.96, p = prevalence, e = tolerable error =5%. A proportion of 20% was used as p (14).

Data collection

With the aid of a pretested questionnaire, demographic data including age, education level, gravidity and gestational age of pregnancy were collected. Pregnant women were also interviewed on awareness of UTI and its complications during pregnancy as well as on other risk factors for UTI. Each participant was provided with sterile container, instructed on the correct mode of self-collection of MSU(write in full before acronym) sample and the importance of a clean catch of MSU. Aliquots of the collected

samples were microscopically examined for bacteria, pus and ova. Rapid urine dipstick test was performed instantly on receipt of the MSU for immediate medical intervention for pregnant women with significant AUTI. Using a calibrated loop (10μl), urine samples were inoculated on Cysteine lactose electrolyte deficient agar (CLED) and incubated overnight. All urine samples that were not cultured within 2 hours were stored at 4°C.

Isolation and identification of bacteria

Colony counts producing bacterial growths of 10^5cfu/ml were considered significant. Bacteria were isolated and identified based on pure colony morphology and biochemical tests (19). Urine samples yielding non-significant or/and mixed growths did not have the test repeated.

Antibacterial susceptibility testing

The Kirby-Bauer disk diffusion method was used for antibacterial susceptibility testing of the isolated bacteria on Muller-Hinton agar plates (LAB-M, Lancashire, UK). Seven antibiotics commonly used for treatment of UTI during pregnancy as per the standard treatment guidelines (STG) of Tanzania were used for this purpose (15). These antibiotics were represented by commercially available sensitivity antibiotic discs namely ampicillin (AP10) discs were used instead), amoxicillin-clavulanic acid (AUG30), nitrofurantoin (NI30), erythromycin (E15), azithromycin (ATH15), ceftriaxone (CRO30) and gentamicin (GM10) (Mast Group Limited, Merseyside, UK). Ceftriaxone and gentamicin were also included as most of the interviewed pregnant women claimed to had previously used them for treatment of UTI. Results were interpreted as susceptible (S), intermediate (I) and resistant (R) as per the Clinical and Laboratory Standard Institute guidelines (20).

Ethical consideration

The present study was approved by the Research and Publication Committee of the Muhimbili University of Health and Allied Sciences, Dar Es Salaam, Tanzania, while written approval letters were obtained from District Medical Officers of the two districts/municipalities. Informed written and verbal consents were obtained from participants before enrollment in the study. For participants under the age of 18 years, consent was also sought from their parents/guardians. The

personal identifiers were removed before data entry and analysis. Results were only disclosed to the willing individual participants and relevant health personnel for medical/treatment purposes.

Data Management

Data were coded and analysed by using the statistical package for social sciences (SPSS version 20) software. Analysis of the prevalence rates of AUTI, antimicrobial resistance (based on zones of inhibition exerted by each tested antibiotic on isolated bacteria) and other investigated parameters were performed. The Dunnett's test (two-sided) was used to compare the significance of differences between zones of inhibition produced by each uropathogenic bacteria to that of control bacterial strains. Univariate analysis of variance, cross-tabulation (Chi-squares) and logistic regression were used for the analysis of various variables such as risk factors for AUTI during pregnancy. Differences among the tested variables were considered significant when $p < 0.05$.

RESULTS
Demographic characteristics of the study population

A total of 202 pregnant women from 50 different localities of Dar Es Salaam City were recruited in this study, their ages ranged from 15-45 years with a median of 26+ years. Slightly more than half (56.9%) of the participants were of ages between 20 and 29 years. Over one third (36%) of participants were primigravida. One hundred and one (50%) participants had primary education. Presence of AUTI among the pregnant women, revealed no direct association with age, education level, gestation age or gravidity (Table 1).

Prevalence of UTI among the study pregnant women

Mid-stream urine (MSU) sample was collected from each participant and subjected to microbiological analysis. About 23% (47) of MSU samples showed significant AUTI (10^5cfu/ml) as shown in Tables 1 and 2. Out of 47 pregnant women with significant AUTI, 21.3% (n=10) and 70.2% (n=33) were in their first and second trimesters respectively (Table 1). The most frequently isolated bacteria was *Eschericia coli* (46.8%) while the least frequently isolated was *Pseudomonas aeruginosa* (2.1%) - Table 2. When results from the MSU culture were compared to those obtained from the rapid

dipstick test; the latter had a sensitivity of 79.2% and specificity of 63.6% with positive and negative predictive values of 40.45% and 90.7% respectively. The urine culture tests yielded positive results in 23.3% of the tested MSU samples, while the dipstick test produced 9.3% false negative results. Odds of the standard culture media (aerobic bacterial counts) yielding positive results was 6.083 higher than that of dipstick test (p< 0.01).

Antimicrobial susceptibility profiling

Our findings revealed high rates of antibacterial resistance among the isolated bacteria, ranging from as high as 86.4% to 100% and 72% to 100% against erythromycin and ampicillin respectively as indicated in Table 3. Most of the tested *E. coli* were resistant to ampicillin (72.7%) and erythromycin (86.4%), while only a few exhibited resistance to nitrofurantoin (18.2%) and azithromycin (36.4%). *Klebsiella pneumoniae* exhibited a high antibacterial resistance against erythromycin (94.7%) while low resistance was observed against gentamicin (5.3%). Three species of bacteria (*Staphylococcus aureus*, *Proteus* species and *Pseudomonas aeruginosa*) were sensitive to nitrofurantoin, but 100% resistant to ampicillin and erythromycin. Azithromycin, ceftriaxone and gentamicin were relatively effective against most of the isolated bacteria, particularly *Pseudomonas aeruginosa* and *Proteus* species. None of the isolates were sensitive to all tested antibiotics (Table 3).

Analysis of some risk factors for AUTI in pregnancy

There was no association between the prevalence of AUTI and gestation age (p = 0.417), educational level (p = 0.383) and gravidity (p = 0.873) of the participants. Cross-tabulation (Chi-squares test) showed no significant association between the prevalence rates of AUTI and the pregnant women's awareness of the UTI-associated complications during pregnancy (p = 0.119),Table 4. However, those who had ever experienced dysuria were 4.68 times more likely to contract AUTI (p<0.01). The odds for pregnant women who had ever experienced febrile episodes to yield significant bacterial growth (AUTI) were 4.34 higher than those who had never experienced (p<0.01). Nineteen and 23 respondents could not respond to the questions on whether they were aware of the UTI associated complications or

/and whether they had ever suffered from UTI respectively (Table 4).

DISCUSSION
The study revealed significant AUTI among the pregnant women in the surveyed health centers, with a prevalence rate of 23.3%. The prevalence rates of 6.1-10.9% have been reported from Iran, 7.3% in Ghana and 13% -21% from Tanzania between 2009 and 2010(9,21). A higher rate (55%) was reported from Nigeria (22). One previous study indicated that the prevalence rate might be even higher in resources-limited countries, particularly among persons of lower socioeconomic status (23). However, most of the observed differences in prevalence rates of AUTI, might be due to microbiological techniques that were employed. The authors agree with a previous finding (9) that dipstick test should also be used in conjunction with other urinalysis techniques to avoid false negative results, since the former has lower sensitivity (79.2%) and specificity (63.6%).

In conformity with another previous study (21), *Escherichia coli* and *Klebsiella pneumoniae* were the predominantly isolated microorganisms. The other isolated bacteria include *S. aureus*, *P. aeruginosa* and *Proteus* species that are less common causes of UTI, unless if introduced iatrogenically (17,24). It is now recognized that despite the geographical variability of uropathogens, the predominant microorganisms are Enterobacteriaceae and especially *Escherichia coli* and *Enterobacter* species (22,26).

All isolated *E. coli* were sensitive to gentamicin, and only a few exhibited resistance to ceftriaxone (4.5%), nitrofurantoin (18.2%) and azithromycin (36.4%). Contrary to our findings, Oladeinde (22) found that nitrofurantoin and amoxicillin had poor activity against most of the uropathogenic bacteria. About 94.7%, 78.9% and 73.7% of *Klebsiella* isolates exhibited resistance to erythromycin, amoxicillin-clavulanic acid and ampicillin respectively; notwithstanding being some of the antibiotics of choice for treatment of UTI during pregnancy in Tanzania (15). The single isolate of *P. aeruginosa* showed 100% resistance to erythromycin, amoxicillin-clavulanate and ampicillin. Gentamicin has proved to be still effective for treatment of AUTI in pregnant womeneven though it is known to be nephrotoxic (27). Therefore, gentamicin should be used whenever clinically indicated. In several resource limited countries like Tanzania, there is

limited availability of medications (28, 29), thus obliging prescribers to prescribe whatever is available. The fact that only 57% of tested antibiotics exerted significant antibacterial activities against the uropathogenic bacteria, shows that there is no universal remedy for treatment of UTI during pregnancy.

A number of factors have been ascribed to the high prevalence of AUTI during pregnancy, which include parity, age, previous medical history of UTI, and socio-economic status among others (4,22). The present study shows that level of education , age, gravidity, gestational age and previous history of UTI are independently associated with AUTI, which is in concordance with previous study by Masinde et al.(9). A statistically significant association was observed between an individual patient's susceptibility to UTI with dysuria and febrile episodes as previously observed (2). It was observed that most of the pregnant women with significant AUTI were in their first and second trimesters with a prevalence of 33.3% and 24.3% respectively. Presumably this is because a number of pregnant women register at ANC during such periods for ascertaining their pregnancy or/and delivery period.

CONCLUSION

This study shows a relatively higher prevalence rate of AUTI (23%) among pregnant women in Dar Es Salaaam as compared to that of previous studies (21%). High antibiotic resistance rates (73-100%) were exhibited by the isolated uropathogenic bacteria. The predominant microorganisms associated with the infection were *E. coli* and *K. peumoniae*. Majority of the isolated bacteria were resistant to erythromycin, amoxicillin-clavulanic acid and ampicillin. Given these observations, we suggest improvement of awareness on local antimicrobial resistance patterns among prescribers in order to facilitate proper antibiotic prescribing. The study also highlights the need for ANC personnel to educate pregnant women on the potential risks of UTI and how to prevent it during their visits to ANC. The authors recommend performing routine urine culture because of the revealed lower sensitivity of rapid dipstick test.

Conflict of interest: The authors declare no conflict of interest.

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Table 1: Demographic characteristics of pregnant women screened for bacteriuria (AUTI)

Investigated Variables		No bacteriuria (%)	Significant Bacteriuria (%)	p-value
Age group	15-24	57(28.2)	17(8.4)	
	25-34	73(36.2)	22(10.8)	
	35-44	25(11.9)	8(4.0)	
	= 45	1(0.5)	-	0.80
Education level	Informal	3(1.5)	2(1)	
	Primary	75 (37.1)	26(12.9)	
	Secondary	65(32.2)	18(8.9)	
	College/University	12(5.9)	1(0.5)	0.415
Gravidity	1-2	97(48)	29(14.3)	
	3-4	51(25.2)	16(8.0)	
	=5	7(3.5)	2(1.0)	0.873
Gestation age	1st trimester	20(10.0)	10(5.0)	
	2nd trimester	103(51.0)	33(16.0)	
	3rd trimester	32(16.0)	4(2.0)	0.093

Table 2: Aerobic bacterial counts of isolated uropathogenic bacteria

Bacterial isolates	Number of pregnant women with AUTI	
	=100000	>100000
None	154 (76.2)	0(0.0)
E. coli	1(0.5)	21(10.4)
K. pneumonae	0(0.0)	19(9.4)
S. aureus	0(0.0)	4(2.0)
Proteus spp	0(0.0)	2(1.0)
P. aeruginosa	0(0.0)	1(0.5)
Total	155(76.7)	47(23.3)

Table 3: Prevalence rate of antibacterial resistance among isolated bacteria

Bacterium	Rates (%) of antibacterial resistance						
	AMP	AMC	NIT	ERY	AZI	CEF	GEN
E. coli	72.7	50	18.2	86.4	36.4	4.5	-
K. pneumoniae	73.7	78.9	36.8	94.7	36.8	10.5	5.3
Staphylococcus aureus	100	75	-	100	25	25	25
Proteus spp	100	50	-	100	-	-	-
P. aeruginosa	100	100	-	100	-	-	-

Keys:AMP-ampicillin; AMC- amoxicillin-clavulanic acid; NIT-nitrofurantoin; ERY-erythromycin; AZI-azithromycin; CEF-ceftriaxone; GEN-gentamicin.

Table 4: Comparative analysis of risk factors for UTI during pregnancy

Variable		Urine culture results		X^2- value	P -value
		Positive	Negative		
Awareness on UTI	Yes	37	125	0.042	0.492
	No	10	30		
Knowledge on complications	Yes	9	41	1.862	0.119*[1]
	No	37	96		
Ever suffered from UTI	Yes	32	87	1.022	0.205*[2]
	No	12	48		
Had febrile episodes	Yes	23	26	19.182	0.001
	No	24	126		
Experienced dysuria	Yes	20	19	24.245	0.001
	No	27	136		

(*[1] and *[2])- a total of 21 and 23 respondents respectively, could not answer the questions.