Antibiotic profile for Staphylococcus aureus and coagulase-negative Staphylococci isolates from pregnant women with asymptomatic bacteriuria in Nairobi, Kenya

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Abstract

Background: Urinary tract infection (UTI) is a condition, which manifest in various forms such as acute, uncomplicated bacterial pyelonephritis, complicated UTI, recurrent cystitis and asymptomatic bacteriuria. Urinary tract infection (UTI) is one of the most frequent types of bacterial infections. The main purpose of this study was to determine the frequency and antibiogram profile of Staphylococcal spp in urine obtained from pregnant women. This was a cross-sectional study involving 1020 women attending antenatal clinic selected clinics in Nairobi County. The midstream urine samples were collected and subjected to culture, and sensitivity. The antimicrobial susceptibility profile of Staphylococcus aureus and (CoNS) strains was done using disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) criteria. 11 antibiotics were used as follows Kanamycin, Gentamycin, Chloramphenicol, and Ampicillin, amoxicillin/clavulanate, Ceftriaxone, Tetracycline, Cotrimoxazole, Ciprofloxacin, Cefotaxime and Imipenem. During the study 219 urine samples were found to have significant asymptomatic bacteriuria. They were the most prevalent aetiological agent in this study. Routine screening for gram positive bacteria as etiological agent of ASB should be considered. The MAR index reported in this study is an indication the isolates were from high risk environment.

Key Words: Antibiogram, Staphylococcus, pregnant women

Introduction

Urinary tract infection (UTI) is a heterogeneous disease, which can be divided into several types of infection, such as acute, uncomplicated bacterial pyelonephritis, complicated UTI, recurrent cystitis and asymptomatic bacteriuria. Urinary tract infection (UTI) is one of the most frequent types of bacterial infections(Nicolle, 2015). Asymptomatic bacteriuria refers to the presence of bacteria in urine. It is a condition in which urine culture reveals a significant growth of pathogens that are greater than 10^5 bacteria/ml, but without the patient showing symptoms(Delzell and Lefevre, 2000).

Staphylococcus aureus infections are increasing in pregnant and postpartum women. S. aureus has been reported to colonize the vagina in 4%-22% of pregnant women(Chen et al., 2006). The major causative agents of UTIs are Escherichia coli and other Enterobacteriaceae. Gram-positive bacteria may be easily overlooked due to limited culture-based assays typically utilized for urine in hospital microbiology laboratories. Some UTIs are polymicrobial in nature, often involving one or more Gram-positive bacteria (Kline and Lewis, 2016). While the uropathogenic agents vary depending upon age, sex, catheterization and hospitalization. The pathogens traditionally associated with UTI are changing many of their features due to development of antimicrobial resistance and other mobile factors. Therefore, complicated UTI has a more diverse etiology than uncomplicated UTI. However organisms that rarely cause disease in healthy patients can cause significant disease in hosts with anatomic, metabolic, or immunologic underlying conditions(Ronald, 2003). Pregnancy increases risks for urinary tract infection due to the dilatation of the ureters from week 6 of pregnancy to delivery. During pregnancy there is increased bladder volume and decreased bladder tone, along with decreased ureteral tone and increased urinary stasis. All these are factors that would increase the risk of development of infection(Nicolle, 2005). Staphylococcus spp are not considered as typical uropathogen, although S. aureus UTI is known to occur in patients with urinary catheters and is associated with the development of invasive infection in these patients. However there is an increasing trend where many studies report dominant etiological agents being either E.coli or S. aureus(Turpin et al., 2007). S. aureus is a worldwide pathogen whose natural reservoir is human and causes severe community-associated infections of skin and soft-tissue (Nordmann and Naas, 2005).

Most asymptomatic bacteriuria (ASB) is caused by bacteria and can easily be treated by antibiotics. However there is increased development of microbial resistance to these antibiotics. Treatment of infections caused by S. aureus can be a challenging experience in clinical practice. S. aureus is known to acquire resistance to new drugs and continues to challenge attempts to clear them during treatment (Onanuga et al., 2005). The resistance of S. aureus isolates to commonly used antibiotics in different parts of the world has been widely reported. Assessment of resistant patterns of microbes to drugs has shown a rise in the incidence of microbial resistance to most prescribed antibiotics(Akortha and Ibadin, 2008).
Therefore this study is aimed at determining the prevalence of Staphylococci spp in ASB cases and also determines their antibiotic. Resistance to antimicrobial agents among gram positive cocci clinical isolates is higher than community isolates (Andrade et al., 2006). Emerging resistance bacterial strains and antimicrobial resistance rates among pathogens recovered from community-acquired urinary tract infections is on the rise. This is being experienced in different magnitude in different regions resulting into limited therapeutic options. This change in microorganisms involved in ASB requires continuous and regular antimicrobial resistance surveillance in order to guide the treatment regimes to be instituted. Most studies on UTIs have concentrated on the antimicrobial resistance profile of Gram-negative enterobacteriaceae especially E. coli which are known to be the most prevalent UTIs causative organism. While the resistance profile of isolated Gram-positive organisms such as S. Aureus are not treated with expected importance despite the increasing prevalent rate of this organism in UTIs and their role in antibiotic resistance.

Sample size

The formula used for sample size calculation was \( n = \frac{z^2 (p(1-p))}{e^2} \) (Sundar, 2004). This study used an estimate of the proportion of population falling into the group of interest at 50%. The prevalence of asymptomatic bacteriuria in pregnancy in low socio-economic population and with specific inclusion criteria is unknown in Kenya. This gave the minimum sample size at 384 however due large patient numbers that are attended at the clinics of interest the sample size was increased for better representation.

Ethical clearance

Ethical clearance for this study was obtained from the KEMRI Ethics Clearance Committee.

Methods

Nairobi, Kenya’s capital city is a typical sub-Saharan Africa (SSA) urban centre characterized by population explosion with a current population of about 3.4 million in 2010. Majority of resident estimated at 60–70% of her population live in informal settlements (UN-Habitat, 2008). The Nairobi county health clinics are distributed within the county and some cater for specialized needs like dental, skin and infectious conditions. Among the health clinics there are those that cater for antenatal clinic and there after normal delivery. These are the ones we selected according to their location in the informal settlement area which are known risk factor for asymptomatic bacteriuria. The clinics run antenatal clinics on a daily basis and all complicated cases are referred to a specialized county hospital within Nairobi.

Study design

This was a cross-sectional study of pregnant women on their first antenatal clinic visit of the Nairobi county health centres. A questionnaire was used to obtain information from the study participants. The information obtained consisted of identification number, age, phone number, educational qualification, marital status, parity, gestational age, and human immune deficiency status. The inclusion criteria involved the first visits of apparently healthy pregnant women attending clinic for the first visit and those who gave their informed consent to participate in the study. However, the women excluded from the study were those who had features of urinary tract infection, fever, had taken antibiotics within 2 weeks of the study, had chronic medical conditions (HIV) retroviral disease, and those who declined to consent despite adequate counselling.

All pregnant women at the antenatal clinic, who met the inclusion criteria, were counselled on how to collect midstream urine. This involved initial instructions by the female attending trained nurses and laboratory technicians. The laboratory technicians supervised the urine-sample collection. The first part of the urine was voided, and approximately 10–15 mL of midstream urine was collected in a sterile universal bottle, that had been correctly labelled and given to the patients. The urine samples in the sterile universal bottles were taken to the laboratory for processing within 1 hour. These samples were subjected to culture, and sensitivity according to standard. Microscopy was done at the clinic involved centrifugation of approximately 10 mL of urine sample in a tube at 1,500 rpm. The sediments were poured on a clean slide and observed under a microscope for casts, pus cells, and yeast cells. The remaining urine was sent to processing laboratory for culture Samples were cultured on air dried plates of Cysteine lactose electrolyte deficient agar (CLED) using a calibrated loop delivering 0.002ml of urine. Plates were incubated aerobically at 37ºC overnight. Colony counts yielding bacterial growth of 10³ organism/ml or more of pure isolates were deemed significant. Contaminated urine usually has less than 10⁴ organism/ml and often contains more than one bacteria species (Cedic Mims et al., 2005). Suspected bacterial species were characterised by colonial morphology, gram stain followed by microscopic examination, motility test and biochemical tests. Isolates were identified to species level using standard methods according to Clinical and Laboratory Standard Institute Guidelines (Clinical Laboratory Standards Institute., 2015). Identification of gram positive cocci isolates was performed by Gram staining, catalase, mannitol fermentation and coagulase tests (Forbes A.B et al., 2007; Mandell et al., 2010).

Antimicrobial susceptibility testing

To evaluate antimicrobial susceptibility of isolates, Kirby- Bauer’s Disk diffusion method was done according to Clinical Laboratory and Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards)(Clinical Laboratory Standards Institute., 2015). The following antimicrobial agents were used in this study: Kanamycin, Gentamycin, Chloramphenicol, and Ampicillin, amoxicillin/clavulanate, Cefazidime, Tetracycline, Cotrimoxazole, Ciprofloxacin, Cefotaxime and imipenem. Briefly, the bacterial suspensions were obtained from overnight cultures. The turbidity of each bacterial suspension was adjusted equivalent to a no. 0.5 McFarland standard and then inoculated on Mueller- Hinton agar (Oxoid, UK). Diameter of inhibition zones was measured after incubation at 35ºC for 18-24 hours, and data was reported as susceptible, intermediate, and resistant. Staphylococcus aureus ATCC 25923 was used as reference strains for susceptibility testing. Multiple Antibiotic Resistance (MAR) index value of all the strains was calculated. The MAR index applied to a single isolate is defined as \( a / b \), where \( a \) represents the number of antibiotics to which the isolate was resistant and \( b \) represents the number of antibiotics to which the isolate
and 11 isolates were resistant to six and seven antibiotics. The age of the women ranged between 16 to 45 years. Staphylococci spp were the most common isolates at 42.9% followed by Escherichia coli (38.8%), then Klebsiella (7.8%), Pseudomonas spp (2.7%), Proteus spp (2.7%), Citrobacter (2.3%), Enterococcus (1.9%) and Enterobacter (0.9%) respectively as indicated in table 1. The resistance and susceptibility pattern of gram positive isolates from positive asymptomatic bacteriuria is shown in table 2. Gram positive bacterial isolates showed a high resistance to ampicillin and cefotaxime ranging from 50% to 92.3%. Imipenem had the lowest resistance to all isolates ranging from 0% to 10.3% and most isolates were susceptible to imipenem ranging from 89.7% to 100%. The resistance to kanamycin was relative low ranging from 20.7% to 24.6% for Proteus spp.

The prevalence of asymptomatic urinary tract infection among pregnant women attending antenatal clinic at Nairobi County antenatal clinics was found to be at 21.5%. This has been reported and discussed in a manuscript published by Pan Africa Medical Journal (Ayoji et al., 2017). The bacteria isolated from urine cultures were Escherichia coli (38.8%) Staphylococcus aureus was (29.7%), Pseudomonas spp was (7.8%), Staphylococcus spp was (2.7%), Proteus spp was (2.7%), Citrobacter spp was (2.3%), Enterococcus spp was (1.9%) and Enterobacter spp was (0.9%) respectively. In overall staphylococcal spp were predominant isolates in this study at 42.9%. This was in agreement with a study done by Tadesse which reported prevalence of staphylococcal spp at 45.6% (Tadesse et al., 2014). The predominance of staphylococcal spp has also been reported by Rahimkhani in a study done in Iran that reported staphylococcal spp at 36% (Rahimkhani et al., 2008). This also agreed with another study done in Nigeria which worked with patients with UTI symptoms. They reported staphylococcal spp as being predominant (Ekwealar et al., 2016). Although the conditions of study were quite different from those of the current study, the significance of staphylococcus spp as an important uropathogen cannot be overlooked.

The prevalence proportion of staphylococcal at 42.9% in this study and similar reports demonstrated by other studies (Ekwealar et al., 2016; Rahimkhani et al., 2008; Tadesse et al., 2014) is an indication that etiological agents for bacterial UTI are dynamic and change with different influencing factors. Gram-positive bacteria are a common cause of urinary tract infection (UTI), particularly among individuals who are elderly, pregnant, or who have other risk factors for UTI (Kline and Lewis, 2016). However urinary tract infections involving Gram-positive bacteria may be easily overlooked due to limited culture-based assays typically utilized for urine investigation in hospital microbiology laboratories (Kline and Lewis, 2016). Experimental studies have demonstrated that
staphylococcal spp are cleared quickly from the bladder however they can co-infect patient with other organisms(Kimberly A. Kline et al., 2010). There is also evidence that Gram-positive bacteria are important uropathogens in their own right (Mcaggart et al., 1990). However they are easily overlooked because they are missed by routine diagnostic methods (Domann et al., 2003)

Effective treatment of patients with UTIs generally relies on the identification of the type of organisms and the selection of an effective antibiotic agent to the organism in question. The pattern of antimicrobial resistance of bacteria producing UTI varies in different regions (Beyene and Tsegaye, 2011). Multidrug-resistant (MDR) was defined as resistance to at least two or more antibiotics (Beyene and Tsegaye, 2011; Esmaeili, 2003). Of 98 isolates tested all (100%) were MDR. In particular, seventeen (18.1%) of isolates were resistant to at least three drugs, another seventeen (18.1%) of isolates were resistant to at least four drugs and sixteen (17.0%) of the isolates were resistant to five drugs. The predominant resistance patterns among the isolates involved Ampicillin, tetracycline, and cotrimaxazole which were reported in 33(56.9%) of the patterns. This was with combination with other antibiotics. The isolates expressed 58 different antibiotic resistant patterns. The susceptibility profile of the isolated bacteria showed wide range of antibiotic resistance patterns.

The data from our investigation exhibited varying antibiotic susceptibility profile with a significant resistance of more than 50% reported for staphylococcus spp isolates to ampicillin, tetracycline, cotrimoxazole, cefazidime and cefotaxime. In our study the susceptibility of staphylococcus spp to Chloramphenicol, ciprofloxacin, gentamycin imipenem and kanamycin ranged from 63.1% to 92.3%. This agreed with other studies done earlier which reported high sensitivity to these antibiotics (Khoshbakht et al., 2012; Kumar .R .et al., 2014). The possible reason for the steady increase in resistance to these antibiotics could be due to sustained selective pressure due to their widespread use. A significant increase in resistance of bacteria causing UTI to, Ampicillin, cephalosporins and cotrimoxazole has been reported worldwide(Gupta et al., 2001). A high incidence of MDR strains was reported in this study. It could be attributable to high usage of antimicrobials agents in the community. Continued use of antibiotic for treatment of UTI and other infection should be monitored to prevent the spread of resistant isolates and also minimize the use of antibiotics for a prolonged period(Mansour et al., 2009).

Gram positive bacterial isolates showed a high resistance to ampicillin and cefotaxime ranging from 50% to 92.3%. Imipenem had the lowest resistance to all isolates ranging from 0% to 10.3% and most isolates were susceptible to imipenem ranging from 89.7% to 100%. The resistance to kanamycin was relative low ranging from 20.7% to 24.6% for staphylococci spp. Staphylococcal spp showed high resistance to ampicillin and cefotaxime while kanamycin and Imipenem reported a relatively higher sensitivity to the bacterial isolates. Twelve (13.3%) of the staphylococci isolates were resistant to more than seven antibiotic tested. This agreed with studies that have demonstrated staphylococci being resistant to penicillins, carbapenems and beta-lactam/beta-lactamase inhibitor combinations (Clinical Laboratory Standards Institute., 2015). Recently, several studies have shown that the methicillin-resistant staphylococci have started to gain resistance to many widely used antibiotics quinolone, macrolide, amino glycosides, tetracycline, cotrimoxazole and Chloramphenicol (Knauer et al., 2004). This study did not report the methicillin-resistant staphylococci however the resistance patterns reported in this study related closely those reported earlier.

Carbapenems are broad-spectrum antibiotics with activity against both Gram-positive organisms and Gram-negative organisms. Seven isolates reported resistance to imipenem which is a carbapenem and they are used as last line antibiotics. Despite introduction of antimicrobial agents and medical improvements in controlling staphylococci infections, they have remained important hospital and community pathogen (Anunit et al., 2016). Furthermore, these bacteria have become a major concern to the medical community due to their extraordinary ability to adapt rapidly to antibiotic stress(Livermore, 2000). Although resistance to imipenem and Kanamycin has been reported among our isolates, however the level of reported was relative low therefore they can be effective drugs for treatment of UTI associated with gram positive cocci. According to our findings, Ampicillin, Cefotaxime, ceftazidime and cotrimoxazole are not effective drugs for treatment of UTI. Progressive increase in resistance to these antibiotics and multiple resistances to antibiotics in present study may be related to increased usage of these antibiotics for treatment of UTI and ability of bacterial strains in acquisition of resistance genes.

The MAR index value reported ranged between 0.18 and 1.0. The predominant MAR index was 0.27 and 0.36% both at 18.1% frequency followed by 0.45% at 17.0%. seven isolates were resistant to 2 / 11 of antibiotics tested while 14 and 11 isolates were resistant to six and seven antibiotics respectively their MAR index = 0.54 and MAR index = 0.6. Others gave higher MARI. MARI is a tool that reveals the spread of bacteria resistance in a given population (Ugwu et al., 2009). Any MARI greater than 0.20 implies that the strains of such bacteria originate from an environment where several antibiotics are used (Ehinmidu, 2003; Tambekar et al., 2006, 2006b; Oli et al., 2013). In this study MRI ≥ 0.2 was expressed by 92.5% of the isolates. This implies that a very large proportion of the bacterial isolates have been exposed to several antibiotics and thus has developed resistance to these antibiotics. Similar incidence was reported in the work of Ehinmidu (Ehinmidu, 2003). A similar report was demonstrated by Prakash and Saxena (Prakash and Saxena, 2013). The high MARI from urine samples isolates in this locality emphasizes the need for continuous monitoring of antibiotic susceptibility profile of bacteria implicated in UTI prior to antibiotic prescription

CONCLUSION

There was a high prevalence of Staphylococcus spp as etiological agents of ASB among pregnant women. Therefore, routine screening for gram positive bacteria as etiological agent of ASB should be considered. The high level of resistance among gram positive bacteria causing ASB limits the use of antimicrobial agents for therapy and also the spread of MDR isolates is a threat to medical practice. Continuous Surveillance for multidrug-resistant strains is necessary to prevent the further spread of resistant isolates.
The MAR index reported in this study is an indication that the isolates frequently subjected to selective pressure.

Acknowledgements

The authors would like to thank the staff of all attending nurses and laboratory scientists from Kangemi, Kahawa and Riruta health centers for their role in evaluation of subjects and collection of samples. The urine microscopy was done at clinics and was done by laboratory scientists. This study was funded by National Commission for Science Technology and Innovation-Kenya.

Reference


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