



Antimicrobial Susceptibility Pattern of Asymptomatic Bacteriuria Isolated from Sickle Cell Disease Patients in Edo State, Nigeria

^{1*}Moses-Otutu, I. M. and ²Okojie, R. O.

¹Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

²Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria

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Abstract

Sickle cell disease (SCD) patients are prone to frequent crisis arising from secondary complications from bacterial, fungal or viral infections. This study is aimed at determining the antimicrobial susceptibility pattern of asymptomatic bacteriuria isolated from SCD patients in Edo State, Nigeria. A total of 185 SCD patients recruited for this study were given sterile universal containers to produce 'clean catch' midstream urine after their informed consent were sought and their medical history obtained from the clinicians. The urine samples were viewed macroscopically, cultured and incubated aerobically at 37°C for 24hrs. The culture media used were CLED and blood agar. Antimicrobial susceptibility test was carried out on all bacteria isolated as stated by Bauer and colleagues. A prevalence of 7.0% asymptomatic bacteriuria (ASB) was obtained among the study participants. ASB was more prevalent in females (84.6%) than males (15.5%). Age group 10-19 years had the highest ASB prevalence (46.1%) while age group >30 years recorded the least prevalence of ASB (7.7%). The bacteria isolated were *Escherichia coli* (30.8%), *Staphylococcus aureus* (23.8%), *Klebsiella* and *Enterobacter* species each were 7.7% respectively while *Candida* species was also isolated. A total of nine (9) antibiotics were used. All isolates were susceptible to cefuroxime and ofloxacin. The Gram negative bacteria were sensitive while the Gram positive bacteria were resistant to amoxicillin clavulanate. All isolates were resistant to the aminoglycosides, gentamicin. Prevention of infection and early detection of ASB is a major way of reducing the frequent crisis and complications arising from bacterial infections in SCD patients.

* Corresponding Author: Moses-Otutu I. M; ifueko.moses-otutu@uniben.edu

Introduction

Infection is the leading cause of morbidity and mortality in Sickle Cell Disease (SCD) patients particularly those living in less developed countries of the world. This is primarily due to the fact that the pattern of morbidity and mortality are less well defined and implementation of preventive care is grossly inadequate (Booth *et al.*, 2010). The sickle gene predisposes SCD patients to an increased susceptibility to infection more especially to bacteria pathogens, the infection then provokes a series of SCD-specific pathophysiological changes (Atkins *et al.*, 1997). In SCD susceptibility to infection, the spleen plays a major role in increased susceptibility to some type of bacterial infections. The major infections occur early in infancy when the spleen is functioning partially (Tamouza *et al.*, 2002).

The presence of bacteria in freshly voided urine is known as bacteriuria. Bacteriuria is considered significant if the bacteria count in the urine is greater than 10⁵ organisms or more per ml 10⁸/l in pure culture (Cheesbrough, 2000; Juthani-Mehta, 2014

and Vasudevan, 2014). Significant bacteriuria can be symptomatic or asymptomatic. Symptomatic bacteriuria is also known as urinary tract infection and is usually accompanied with varied symptoms such as increased number of pus cells in urine (pyuria), sometimes increased number of red blood cells in urine (haematuria), pain on passing urine (dysuria), suprapubic pain and frequent urination (Vasudevan, 2014). On the contrary, in asymptomatic bacteriuria (ASB), the varied symptoms of urinary tract infection are absent. Alterations in host-pathogen interaction are thought to be responsible for the absence of symptoms despite the presence of urinary pathogens in asymptomatic bacteriuria (Ragnarsdottir and Svanborg, 2017). Sickle cell disease patients, diabetics, the elderly and catheterized bladders are prone to acquiring asymptomatic bacteriuria (Mims *et al.*, 1990; Nicolle *et al.*, 2005 and Ariathianto, 2011).

SCD has a prevalence of about 2 to 3% in the Nigerian population of more than 160 million people and a prevalence of

2.39% in Benin City, South-South Nigeria with an SCD carrier rate of about 23% (Akinyanju, 1989; Odunvbun *et al.*, 2008 and Nwogoh *et al.*, 2012). SCD patients are burdened with increased frequency and severity of infections particularly with encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae* (Fixler and Styles, 2002). Also commonly isolated pathogens from SCD patients include *Escherichia coli*, *Proteus* species, *Klebsiella* species, *Enterococcus faecalis* and *Staphylococcus* species (Ozturk *et al.*, 2006). Asymptomatic bacteriuria can progress to pyelonephritis and renal function impairment in immunocompromised patients such as SCD patients and pregnant women (Nicolle, 2003 and Schnarr and Smaill, 2008). Early detection of infections is the key, thus enabling initiation of treatment to reduce complications. This study examines antimicrobial susceptibility pattern of asymptomatic bacteriuria isolated from sickle cell disease patients in Edo State, Nigeria.

Materials and Methods

Study Population

This study was conducted at the Edo State Sickle Cell Center, Benin City, Nigeria. The Edo State Sickle Cell Center has a referral status for the management of SCD patients in Edo State and neighboring Ondo State (west), Delta State (south), Anambra State (east) and Kogi State (north).

The study population consisted of Sickle Cell Disease patients aged 2years to 49years whose genotype were previously confirmed by cellulose acetate electrophoresis to be homozygous haemoglobin SS, attending the clinics at the Sickle Cell Center. A total of 185 SCD patients were assessed for this study.

SCD patients who were in the clinic with SCD crisis and complaints suggestive of symptomatic UTI (such as dysuria, suprapubic pain, loin pain, frequent urination and urgency), SCD patients who had been on antibiotics in the two weeks preceding recruitment for this study and SCD patients currently on antibiotics were excluded from this study.

Ethical consideration

Ethical clearance was obtained from the Ethics and Research committee, Ministry of Health, Edo State, Nigeria. The sample collection method was explained to each participant using the informed consent form and questionnaire prepared by the researcher. Those that consented to participate in the study were made to sign the informed consent form before being eligible to participate in this study while parents signed the consent form on behalf of their children. Permission from the coordinators of the Sickle Cell Disease Centre Benin City, Edo State, Nigeria was also obtained.

Sampling method and analysis

Each participant was given a well labeled, boric acid containing, sterile, dry, wide-mouth universal container and asked to produce 10-20ml 'clean-catch' midstream of the first urine passed by the patient at the beginning of the day. The samples were later collected and then taken to the laboratory for analyses.

Urine Microscopy

Urine macroscopy was carried out to report the appearance of each urine sample. Each urine sample was properly mixed by gentle inversion; an aliquot of 10ml was aseptically transferred into a test tube and centrifuged at 1000rpm for 5min. The supernatant was discarded; the urine sediment was remixed by gently tapping the test tube and then transferred into a glass slide, then covered with a cover slip and examined microscopically for the presence of pus cells, yeast cells, red blood cells cast, crystals, bacteria and possible parasites per high power field.

A standard wire loop of 2 mm internal diameter, 6cm long and delivering 0.002 ml per loopful of urine was sterilized on a Bunsen flame, immersed in a properly mixed uncentrifuged urine and streaked on dried CLED and 5% Blood agar media in order to obtain the growth of both Gram-negative and Gram-positive organisms. The plates were then incubated at 37°C for 24hrs. After 24hrs, the plates were read. The bacteria colonies were then counted before multiplying by 100 to give an estimate of the number of bacteria present per millimeter of urine. Samples that yielded pure bacteria growth of 100000/ml (10^5 /ml) or more were recorded as yielding significant bacteriuria. Samples yielding 10 000-100 000/ml (10^4 - 10^5 /ml) bacteria counts were repeated while counts less than 10 000/ml (10^4 /ml) organisms /ml were recorded as insignificant bacteria growth. The repeated urine samples that yielded significant bacteriuria were regarded as having asymptomatic bacteriuria. Gram staining reactions was also carried out for cell morphology. Mixed growth or growth of more than one species of organism in an agar plate (growth such as that of normal skin flora) were regarded as contaminants and therefore disregarded. Biochemical identification tests namely motility, catalase, coagulase, citrate utilization test, litmus milk decolourization, indole and beta-glucuronidase test were used to identify the organisms (Cheesbrough, 2000). The isolates were categorized based on a combination of their Gram staining reaction, cultural, morphological and biochemical characteristics. Antibiotic susceptibility test was done using the disc diffusion method, a modified method of Bauer *et al.* (1966). Pure culture of the bacteria isolates were used to obtain turbidity comparable with 0.5% McFarland's standard solution. 2-3loopfuls of the suspension was streaked evenly on already dried nutrient agar plates. Aseptically, appropriate multi disc containing minimum inhibitory concentrations (MIC) of

Cefuroxime (30µg), Cefixime (5µg), Gentamycin (10µg), Ofloxacin (5µg), Ceftazidime (30µg), Amoxicillin/Clavulanate (10µg), Cefuroxime (30µg), Ciprofloxacin (5µg), and Tetracycline (25µg) were firmly placed on the surface of the dried nutrient agar plates. The multi disc containing Nalidixic acid was used for the Gram negative bacteria. The plates were then inverted and incubated at 37°C for 24hrs. After 24hrs incubation, the clear zones that developed around each disc were measured in millimeter (mm). The zone of inhibition of each organism to each antibiotic was reported as either being sensitive or resistance accordingly. The zones of inhibition were compared with the zones of the control organism. Standard sensitive strain of *E. coli* and *S. aureus* was used as the control organism

Results

The colour of the urine samples ranged from light yellow to dark yellow, some urine samples were pale and some cloudy in appearance. Of the 185 urine samples examined 89 (48.1%) were from male participants and 96 (51.9%) from females. The study participants within the age group 0-9years constituted 48 (26.0%), age groups 10-19years and 20-29years were 77 (41.6%) and 38 (20.5%) while age group >30years constituted 22 (11.9%) (Table 1).

Out of the 185 participants, 10 (5.4%) had significant bacteria in their urine, 13 (7.0%) had asymptomatic bacteriuria (ASB) while 162 (87.6%) had insignificant bacteria count in their urine samples (Table 2).

Four bacterial and one fungal species were isolated from the participants that had ASB and they comprised *E. coli* 4 (30.8%), *S. aureus* 3 (23.0%) while *Klebsiella* species and *Enterobacter* species recorded 1 (07.7%) each respectively and *Candida* species recorded 4 (30.8%) (Table 3).

Table 1: Demographic Parameters of Sickle Cell Disease Patients in Edo State, Nigeria

Variable	No Examined(%)	No positive	% positive
Gender			
Male	89 (48.10)	02	15.40
Female	96 (51.90)	11	84.60
Age (yrs)			
0 – 9	48 (26.0)	06	23.10
10 – 19	77 (41.60)	03	46.10
20 – 29	38 (20.50)	01	23.10
>30	22 (11.90)	03	7.70
Total	185 (100)	13	100

Table 2: Prevalence of Significant Growth and Asymptomatic Bacteriuria among one hundred and eighty- five SCD Patients in Edo State, Nigeria

Variable	No. Positive	% Positive
Significant Growth	10	5.40
Asymptomatic Bacteriuria	13	7.00
No Significant Growth	162	87.60

Table 3: Frequency of occurrence of bacteria and fungus isolated among one hundred and eighty-five SCD Patients

Organisms isolated	No. of organisms is isolated	% occurrence
<i>E. coli</i>	04	30.80
<i>Klebsiella</i> spp	01	07.70
<i>S. aureus</i>	03	23.00
<i>Enterobacter</i> spp	01	07.70
<i>Candida</i> spp	04	30.80
Total	13	100

Table 4: Antimicrobial Susceptibility Pattern of isolated bacteria

Organism Isolated	Source	Sensitivity Pattern (please re-arrange)
Gram Negative <i>E. coli</i>	SCD	Sensitive to: CRX, OFL, LVX, TE, AMX/C Resistant to: GEN, CXM, CIP, CAZ
<i>Klebsiella</i> species	SCD	Sensitive to: CRX, OFL, CAZ Resistant to: GEN, CXM, LVX, TE, AMX/C, CIP
Gram Positive <i>S. aureus</i>	SCD	Sensitive to: CRX, OFL, LVX, TE, CIP, NA Resistant to: GEN, CXM, AMX/C, CAZ
<i>Enterobacter</i> species	SCD	Sensitive to: CRX, OFL, LVX, TE, CAZ, CIP Resistant to: GEN, CXM, AMX/C, NA

Key: CRX-Cefuroxime, OFL-Ofloxacin, LVX-Levofloxacin, TE-Tetracycline, AMX/C-Amoxicillin Clavulanate, GEN-Gentamycin, CXM-Cefixime, CAZ-Ceftazidime, CIP-Ciprofloxacin, NA-Nalidixic Acid

Discussion

A prevalence of 7.0% asymptomatic bacteriuria was obtained among sickle cell disease patients in Edo State, Nigeria. A similar study by Cumming *et al.* (2006), Akinbami *et al.* (2014) and Donkor *et al.* (2017) obtained an ASB prevalence of 10.9%, 44.4% and 17.0% among SCD patients in Jamaica, Lagos-Nigeria and Ghana respectively. Bacteria proliferation in the urine of SCD patients is a common occurrence which is made possible due to the production of abnormally dilute and alkaline urine which results from the altered blood flow in the renal vasculature. This ultimately causes papillary necrosis and loss of urinary concentrating and acidifying capability of the nephrons in some SCD patients (Nicolle, 2003; Schnarr and Smaill, 2008 and Akinbami *et al.*, 2014).

ASB prevalence in the general population is predominantly determined by gender, age and sexual dimorphism. In our study, ASB prevalence was higher in females (84.6%) when compared with their male counterparts (15.4%). Factors that make females more prone to ASB include: the closeness of the urethra meatus to the anus, shortness of the urethra, relative deficiency in secretory Immunoglobulin A, antibody response from mucosal surface in the urogenital tract of females and sexual intercourse (Asinobi *et al.*, 2003 and Chukwu *et al.*, 2011).

Our study recorded ASB prevalence of 46.1% among participants of age group 10-19 years with age group >30 years recording the least prevalence of 7.7%. Major infections in SCD occur early in infancy when the spleen is partially functioning and where additional immune defect is also a contributory

factor despite modern prophylaxis (Tamouza *et al.*, 2002). In normal individuals, ASB prevalence increases with increase in age. The prevalence in women less than 50 years old was 5 - 15% while in men within the age 65-80 years and those aged 80 years and above, the prevalence rate increased from 40% to 50% (Ottolini *et al.*, 1995; Saborio and Scheinman, 1999).

E. coli and *S. aureus* which were the predominant bacteria isolated from the urine samples in our study is in accordance with commonly isolated bacteria from previous studies (Wierenga *et al.*, 2001; Nicolle, 2003). SCD patients just like normal individuals are predisposed to urinary tract infection caused by *E. coli* and some other anaerobes. Urinary tract infections caused by *Klebsiella* species and *S. aureus* are usually associated with hospital acquired infections, often as a result of catheterization

or surgery (Nicolle, 2003). Uropathogenic strains of microbes have developed mechanisms to invade host defense and promote their survival in the urinary space. The mechanisms used by these uropathogenic microbes include the presence of adhesion molecules that aids their attachment to the urogenital tract and the production of α -haemolysin and cytotoxic necrotizing factor 1 (CNF1). (Magnus *et al.*, 1999; Wierenga *et al.*, 2001 and Nicolle, 2003). Candida urinary tract infection is commonly found in individuals with immunosuppression (Nicolle *et al.*, 2003; Almeida and Roberts, 2005; Vasudevan, 2014).

Generally, the cephalosporins are used for the treatment of Gram negative bacteria such as *E. coli* and *Klebsiella* species, Gram positive cocci such as *Staphylococcus* species and most of the Enterobacteriaceae such as *Enterobacter* species.

Moreso, the cephalosporins (cefuroxime and ceftriaxone) are drugs of choice for the treatment of acute chest syndrome in SCD (Fixler and Styles, 2002). The cephalosporins are grouped into various generations as their susceptibility pattern differs a little. In our study, Cefuroxime a second generation cephalosporin had the greatest inhibition activity against all the bacteria isolated. The reason behind this high sensitivity might be because cefuroxime is a highly expensive drug in the Nigerian market and is mostly administered using injections, thus purchase over the counter and abuse is rare. Meanwhile the third generation cephalosporins (cefixime and ceftazidime) used in our study was unable to inhibit all the bacterial isolates, a worrisome outcome. Cefixime, an oral take home drug in SCD treatment might have been frequently abused without the patients completing the required dosage each time it is prescribed.

The two most predominant bacteria isolated in our study were generally susceptible to the fluoroquinolones (ofloxacin and levofloxacin). The fluoroquinolones are antibiotics known to cause serious side effects such as nerve damage or peripheral neuropathy. The fluoroquinolone, levofloxacin is sometimes used in combination with ceftriaxone in the management of acute chest syndrome in SCD patients (Adamkiewicz, *et al.*, 2003). Thus, ofloxacin and levofloxacin are expensive and new fluoroquinolones that are not readily prescribed. *S. aureus* was sensitive while *E. coli* was resistant to this particular fluoroquinolone, ciprofloxacin. Ciprofloxacin used to be a drug of choice that was easily prescribed and could be purchased over the counter; this might have given room to its abuse and subsequent resistance by some bacterial isolates.

All the bacterial isolates obtained in this study were resistant to the aminoglycoside, gentamicin. Though the aminoglycosides are often used in combination with other antibiotics, the outcome in our study is worrisome. Gentamicin could be administered through injections and over the counter purchase is rare. Amoxicillin and Tetracyclin which were previously prescribed, frequently abused and abandoned were able to inhibit the Gram negative bacteria obtained in our study. The penicillin (amoxicillin) is the drug of choice for the prophylactic management of SCD patients under 5 years (Fixler and Styles, 2002 and Adamkiewicz *et al.*, 2003). Amoxicillin used as a prophylactic drug in the management SCD might have become more sensitive with the addition of the clavulanate potassium. The *Candida* species isolated was not subjected to antibiotic susceptibility test as they do not respond to antibacterial agents.

Conclusion

An overall prevalence of 7.0% ASB was obtained among SCD patients in Edo State, Nigeria. All bacterial isolates obtained in this study were sensitive to the second generation cephalosporin, cefuroxime and the fluoroquinolone, ofloxacin. The isolates were all resistant to the aminoglycoside, gentamicin. Prevention of infection and early detection of ASB

is key to reducing frequent crisis as well as complications from bacterial infections in SCD patients. Purchase of over the counter drugs and taking antibiotics without completing the required dosage practice should be discouraged.

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Conflict of Interest

The authors declare that there are no conflicts of interest. MOIM conceptualized the idea and ORO reviewed and handled the final draft.

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