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Bacterial Profile and Antimicrobial Sensitivity Patterns in Asymptomatic Bacteriuria: A Cross-sectional Study of Sickle Cell Disease Patients in the Ho Municipality, Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. Author SFDA designed the study, searched literature, wrote the protocol and collected data. Authors EOA and HA managed the preliminary statistical analysis and wrote the first draft of the manuscript. Authors AAY and PAT managed the final statistical analysis of the study and wrote the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sickle cell disease (SCD) patients are vulnerable to asymptomatic urinary tract infection (UTI), and this can lead to long lasting kidney problems.
Aim: This cross-sectional study assessed the bacterial profile and examined the sensitivity patterns of the isolated bacteria among the SCD patients.

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Methods: From January 2014 to April 2014, Seventy-one (71) patients were consecutively sampled from the sickle cell clinic of Volta Regional Hospital, Ho-Ghana. Mid-stream urine samples were collected for culture and sensitivity. Bacteria isolated were identified and tested for their antimicrobial sensitivity patterns using the Kirby-Bauer disc diffusion method. Independent t-test, Pearson Chi-square test and ANOVA were used to determine mean, standard deviations, associations and differences in groups. P value < 0.05 was considered statistically significant.
Results: The study showed a bacteria profile of *Escherichia coli*, *Staphylococus aureus* and *Citrobacter spp* among the SCD participants. Antimicrobial sensitivity patterns depicted *Escherichia coli* as sensitive to nitrofurantoin and gentamicin while *Citrobacter spp*. was sensitive to Nitrofurantoin. *Staphylococus aureus* was sensitive to cotrimoxazole with all three isolates resistant to ampicillin. 8.5% of the participants had asymptomatic bacteriuria (ASB) and was more in females (66.7%) than in males (33.3%) and in SS genotype (83.3%) than in SC genotype (16.7%).
Conclusion: The research found the prevalence of ASB among SCD patients to be most common in females and SS genotypes. *Escherichia coli* was the predominant isolate and this isolate was susceptible to nitrofurantoin but highly resistant to ampicillin. Urine culture and sensitivity should be included in the clinical assessments of SCD patients and education and awareness on the importance of personal hygiene, particularly in sickle cell disease patients should also be encouraged.

Keywords: Antimicrobial sensitivity; asymptomatic bacteriuria (ASB); bacterial profile; patterns sickle cell disease.

1. INTRODUCTION

Asymptomatic bacteriuria (ASB) can be defined as quantitative growth of bacteria ≥105 colony forming units per millimetres (CFU/ml) urine of the same organism on aseptically collected mid-stream urine (MSU) specimen, in the absence of symptoms of urinary tract infections (UTI) [1,2]. Sickle cell disease (SCD) still remains one of the common groups of inherited disorders in which haemoglobin does not function properly. It is characterized by the inheritance of 2 abnormal haemoglobins of which one is haemoglobin S (HbS). The Haemoglobin S (HbS) is a type of haemoglobin that differs from the normal adult haemoglobin in terms of structure and is inherited as an autosomal recessive trait [3]. According to Grosse et al. [4] Sickle cell disease is common throughout the sub-Saharan Africa, affecting up to 3% of births in some parts of the continent such as Burkina Faso, Nigeria (rural Garki), Ghana, Zambia, Kenya, Tanzania and Gambia and Guinea-Bissau. Sickle cell disease patients usually experience renal dysfunction in the primary stages of their life [5]. Additionally, symptomatic urinary tract infection is associated with painful crises, bacteremia, pneumonia and osteomyelitis in sickle cell disease [6].

Sickle cell disease is a significant health menace with about 200,000 babies born annually with SCD in Africa [7]. Furthermore, about 80% of all children born with SCD are found in sub-Saharan Africa [8]. It has a prevalence of 2% in West Africa and Ghana respectively and is known to usually cause adverse effects on growth, organ impairment as well as renal dysfunction with reports of SCD associated with high morbidity and mortality rate in children with a rate of about 50% - 90% [9,10]. Works by Konotey-Ahulu et al. [11] revealed susceptibility of SCD patients to ASB and other infections than those with normal haemoglobin (Hb). However, there is paucity of data on ASB in patients with SCD. Therefore, this study assessed the bacterial profile and examine the sensitivity patterns of the isolated bacteria among the SCD patients in Ho Municipality, Ghana. The identification of these organisms among SCD patients and the antimicrobial sensitivity pattern will help manage the condition to avoid the development of kidney disease later.

2. MATERIAL AND METHODS

2.1 Study Design/Participants Selection

This was a cross-sectional study with consecutive sampling, conducted amongst sickle cell disease (SCD) patients aged one year to fifty years, attending the Sickle Cell Clinic at Volta Regional Hospital (VRH) in the Ho municipality, Ghana. Seventy-one (71) SCD patients were recruited for the study from January 2014 to April 2014. A well-structured questionnaire was administered to each participant through interview, to obtain information on clinical history and demography. Participants whose genotypes were determined using Gel electrophoresis as

HbSS and HbSC and are in steady states with no signs of urinary tract infections (UTI) and without antibiotic therapy for at least three weeks were eligible for recruitment into the study. Participants with symptoms of UTI, on antibiotics therapy, or in an unsteady state were not studied. Individuals with sickle cell trait (HbAS) were excluded.

2.2 Urine Collection, Culture and Sensitivity (C/S)

About 5 to 10 ml of mid-stream urine (MSU) was collected from the participants into sterile containers and immediately transported to the laboratory for processing. A sterile standard calibrated wire loop (0.02 µl) was used to inoculate 0.02 ml of the well mixed MSU urine onto Cysteine Lactose Electrolyte Deficient agar (CLED) plates. The plates were incubated aerobically at 37ºC for 18-24 hours. Culture plates without visible growth were further incubated for additional 24 hours before being discarded.

2.2.1 Identification and counting of Bacterial Isolates

Bacterial isolates were identified based on a combination of cultural characteristics and morphology (colony size, colour, elevation, edge, surface, opacity, consistency, lactose fermentation, gram reaction, shape and arrangement) as well as biochemical characteristics (catalase, urease, coagulase, indole, motility indole and ornithine and triple sugar iron agar). Bacterial count was determined from the product of the colony count on CLED and loop volume. Bacterial counts >105 CFU/ml was considered significant whilst bacterial count >104 CFU/ml was considered insignificant. Bacterial counts 104-105 CFU/ml was considered doubtfully significant [1].

2.2.2 Antimicrobial Susceptibility Testing (AST)

The Kirby-Bauer disc diffusion technique was employed: antibiotic-impregnated disc (Abtek biologicals) ® was used. Fresh isolates of pure colonies in peptone water were emulsified with a standard sterile loop and turbidity adjusted to 0.5 McFarland Standard. A sterile swab was used to transfer a portion of the emulsified suspension onto the Mueller-Hinton agar surface while rotating the plate. Antibiotic discs was impregnated onto the plate of susceptibility testing agar and the plate incubated overnight at 35-37ºC. The zone of inhibition was determined in millimeters with a caliper and compared to a standard chart to determine susceptibility categorized as sensitive or resistant as previously described by Tagoe and Des-bordes in 2012. A Gram negative-organism *Escherichia coli* (NCTC 10418) and Staphylococcus aureus [National collection of type cultures (NCTC) 6571], a Gram–positive organism, were used as controls.

2.3 Statistical Analysis

Statistical Package for Social Sciences, version 16, SPSS v16 (SPSS, Inc., Chicago Ill) was used for analysis. Variables were reported as mean and standard deviation. Independent t-test, Pearson Chi‑square test and ANOVA were used to determine differences in groups.

3. RESULTS AND DISCUSSION

As depicted in Table 1, mean age was 20.18 ± 12.32. Significant bacterial growth was 8.5% (6) whereas 2.8% (2) revealed insignificant bacterial growth, however 63(88.7%) showed no bacterial growth. Significant bacteriuria was more in females (66.7%) than in males (33.3%) and also higher in SS genotype (83.3%) than in SC genotype (16.7%).

SB was more in the SS patients than in the SC patients (P=0.987). *Escherichia coli* and *Staphylococcus aureus* were the isolates in SS patients whereas *Citrobater spp*. was the only isolate in SC patients Table 2.

Table 3 summarizes the varying susceptibility patterns of the bacterial isolates to antimicrobial drugs. Nitrofurantoin, gentamicin, cefuroxime and pipemedic acid were sensitive to *Escherichia coli* while only nitrofurantoin was sensitive to *Citrobacter spp. Staphylococcus aureus* was sensitive to cotrimoxazole, nitrofurantoin gentamicin and pipemedic acid, however, all the isolates were resistant to ampicillin.

SCD patients are susceptible to asymptomatic bacteriuria (ASB) and other infections due to the hemodynamic changes and immunocom-promised states that occur with anemia and recurrent vaso-occlusion [11]. This study determined the bacterial profile and the antimicrobial sensitivity patterns of the isolates among SCD patients in Ho Municipality, Ghana. The study showed a bacteria profile of *Escherichia coli*, *Staphylococus aureus* and *Citrobacter spp* among the SCD participants. Antimicrobial sensitivity patterns depicted *Escherichia coli* as sensitive to nitrofurantoin and gentamicin while *Citrobacter spp*. was sensitive

to Nitrofurantoin. *Staphylococus aureus* was sensitive to cotrimoxazole and all three isolates resistant to ampicillin. 8.5% of the participants had ASB and was more in females (66.7%) than in males (33.3%) and also higher in SS genotype (83.3%) than in SC genotype (16.7%).

The bacteria profile of ASB observed was similar with previous studies [12,13,14,15]. These studies established a bacteria profile of *Escherichia coli*, *Staphylococcus aureus*, *Citobacter spp*. and *Kebsiella spp* with *Escherichia coli*, the most predominant isolate. The predominance of *Escherichia coli* was consistent with the present study. However, the slight difference in the isolates and percentages of these isolates could be attributed to sample size and the proportion of males and females recruited for the study as well as the SCD genotypes recruited.

The antimicrobial sensitivity pattern from this study was similar to other studies [12,16]. These studies revealed nitrofurantoin, gentamicin and cotrimoxazole as the sensitive antimicrobial agents with ampicillin, the most resistant. However, other resistant antimicrobial agent such as tetracycline and nalidixic acid was observed in the present study. This high resistance was noted by other studies [17,18]. This resistance could be linked to the misuse of such antibiotics which are commonly found over the counter since the choice of antibiotic should be based on urine culture, clinical data and the characteristics of the antibiotic.

Table 1. General characteristics of study participants according to diagnosis of asymptomatic bacteriuria

Variable	Total	CFU/ml			p-value
		Positive	Suspected	Negative	
		≥ 10^5	10^2-10^4	< 10^2	
	(n=71)	(n=6)	(n=2)	(n=63)	
Age (years)					
(Mean ± SD)	20.18 ± 12.32	25.00 ± 12.62	31.00 ± 16.97	19.38 ± 12.13	0.259
Gender (%)					
Male	33 (46.5)	2 (33.3)	0 (0.0)	31 (49.2)	0.310
Female	38 (53.5)	4 (66.7)	2 (100)	32 (50.8)	
Age group(years)					
< 10	15 (21.1)	0 (0.0)	0 (0.0)	15 (23.8)	0.279
10-19	23 (32.4)	2 (33.3)	1 (50.0)	20 (31.7)	
20-29	19 (26.8)	3 (50.0)	0 (0.0)	16 (25.4)	
30-39	8 (11.3)	0 (0.0)	0 (0.0)	8 (12.7)	
≥ 40	6 (8.5)	1 (16.7)	1 (50.0)	4 (6.3)	
SCD genotype					
SS	59 (83.1)	5 (83.3)	2 (100)	52 (82.5)	0.81
SC	12 (16.9)	1 (16.7)	0 (0.0)	11 (17.5)	

CFU = Colony forming units, ml = milliliters, SCD= Sickle cell disease, SD= Standard Deviation

Table 2. Prevalence of bacteriuria among study participants according to SCD genotype

Characteristics	SCD Genotype			
	SS	SC	Total	P-value
	(n = 59)	(n = 12)	(n = 71)	
Bacteriuria status				0.987
SB	5 (8.5)	1 (8.3)	6 (8.5)	
NSB	54 (91.5)	11 (91.7)	65 (9.5)	
Bacterial isolate				0.115
Escherichia coli	3 (60.0)	0 (0.0)	3 (50.0)	
Citrobacter spp.	0 (0.0)	1 (100)	1 (16.7)	
Staphylococcus aureus	2 (40.0)	0 (0.0)	2 (33.3)	

SB = Significant Bacteriuria, NSB = Non-Significant Bacteriuria

Table 3. Antibiotic susceptibility pattern of bacteria isolates causing asymptomatic bacteriuria (ASB) in SCD patients

Antibiotics	Bacteria isolate		
	Escherichia coli	*Citrobacter spp.*	*Staphylococcus aureus*
	(n = 3)	(n = 1)	(n = 2)
AMP	0 (0.0)	0 (0.0)	0 (0.0)
TET	0 (0.0)	0 (0.0)	0 (0.0)
COT	0 (0.0)	0 (0.0)	2 (100)
NAL	0 (0.0)	0 (0.0)	0 (0.0)
NIT	3 (100)	1 (100)	2 (100)
GEN	2 (66.7)	0 (0.0)	2 (100)
CRX	2 (66.7)	0 (0.0)	0 (0.0)
PPA	2 (66.7)	0 (0.0)	1 (50.0)

AMP=Ampicillin, TET=Tetracycline, COT=Cotrimoxazole, NAL=Nalidixic Acid, NIT=Nitrofurantoin, GEN=Gentamicin, CRX=Cefuroxime, PPA=Pipemedic Acid

The prevalence rate of ASB revealed was lower than that reported in other studies [19,20]. In a study conducted by Cumming et al. [19] among SCD children in Jamaica, an ASB prevalence of 10.9% was reported. Furthermore, a recent study by Iwalokun et al. [20] in Lagos, Nigeria reported an ASB prevalence of 14.6%. The inconsistency in prevalence can be attributed to sample sizes, participants recruited for the study and geographical location. However, a recent study by Brown et al. [14] reported a prevalence of 6% in children with sickle cell disease in Enugu, Nigeria. Our study was also consistent with the low prevalence reported by Chukwu et al. [14]. ASB was higher in SCD females than in SCD males. This is supported by a ratio of females to males of 2:1. This ratio is consistent with the ratio 3:2 reported by Ajasin et al. [12] but contradicts reports by Chukwu et al. [14] 5:1 and Tarry et al. [15] 10:1. However, the above ratio supports our findings with bacteriuria higher in females than in males. The difference in ratio can be related to large sample sizes used in the other studies, however, high ratio in females could be attributed to the anatomy of the genitourinary tract system: short course of the urethra and its proximity to the anal region as well as the large surface area of the vagina, which allows easy colonization by bacteria [21,22,23]. ASB was higher in SS genotype than in SC genotype. Hemodynamic changes that occur with chronic anemia and the consequences of recurrent vaso-occlusion often occurs in SS genotype than in SC genotype and this can lead to functional and structural changes contributing to the above observation.

4. CONCLUSION

This study revealed that, ASB is in SCD patients, and females and SS genotypes predominantly affected. *Escherichia coli* was the predominant isolate and the isolates were susceptible to nitrofurantoin but highly resistant to ampicillin. Urine culture and sensitivity should be included in the clinical assessments of SCD patients and education and awareness on the importance of personal hygiene, particularly in sickle cell disease patients should also be encouraged.

CONSENT

Written informed consent was obtained from participants before the study took place and these documents are preserved by the authors.

ETHICAL APPROVAL

Authorities of the Volta Regional Hospital and the Institutional Review Board (IRB) of University of Cape Coast, (UCCIRB) approved the study and these documents are preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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