

Characterization of Bacterial Isolates Cultured from the Nasopharynx of Children with Sickle Cell Disease (SCD)

ORIGINAL

Abstract

Background: We characterized bacterial isolates from the nasopharynx of 84 Sickle cell disease patients 78 of whom were HbSS and 6 HbSC aged 4 four months to 15 years at Wesley Guild Hospital, Ilesa, southwestern Nigeria between February–September, 2014.

Method: Sterile cotton-tipped initially dipped into sterile saline thereafter was introduced into nasopharynx of each patient and thereafter applied onto sterile thioglycolate medium and incubated at 37°C for 24 hr. When growth was noticed, samples were inoculated onto enriched, selective and differential bacteriologic media. Bacterial colonies that grew on such media were picked and characterized by Grams' reaction, cultural, morphologic and biochemical methods. Antibiotic sensitivity tests were determined by the disc diffusion method. Demographic data relating to severity of SCD were provided.

Results: Altogether, 119 isolates were cultured from the nasopharynx. Gram positive bacteria predominated (65.54%) and *Corynebacterium* spp (44.53%) dominated comprising of 19 (35.84%) *Corynebacterium xerosis* 11 (20.75%) *Corynebacterium diphtheriae*, 10 (18.86) *Corynebacterium pseudodiphtheriticum*, 8 (15.09%) *Corynebacterium ulcerans*, 3 (6.66%) *Corynebacterium* spp and 2 (3.77%) *Corynebacterium jeikeium*. Other Gram positive rods cultured were *Arcanobacterium haemolyticum* 6 (5%). *Bacillus subtilis* was 3 (2.5%), *Actinomyces isrealii* 3 (2.5%) and *Norcadia asteroides* 1 (0.84%). Low frequency of nasopharyngeal colonization recorded for *Haemophilus influenzae* 4.2%, *S. pneumoniae* 2.5% and *S. aureus* 4 (3.36%) and multiresistance was widespread for most isolates.

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Conclusion: Indigenous microflora *Corynebacterium* spp predominated and low rate of nasopharyngeal colonization with *H. influenzae*, and *S. pneumoniae* recorded attributable to prophylactic use of penicillin and vaccines administration probably suppressed growth of organisms and inevitably increased resistance to many antibiotics.

Keywords

SCD patients, nasopharyngeal bacterial isolates, Antibiotic resistance, MAR index.

Introduction

A recent report shows about one hundred and fifty thousand infants are born annually with sickle cell disorder in Nigeria [1]. The country also ranks highest in the incidence of sickle cell trait in Africa [1]. According to a recent release by the Sickle Cell Foundation of Nigeria, one out of four Nigerians carries the sickle cell gene compared with the United States of America where one in twelve of African Americans carry the sickle cell gene [1]. It has been shown nasopharyngeal bacterial colonization is common in the young infants which often precedes development of invasive diseases [2]. Knowledge of nasopharyngeal bacteria colonization in children is desirable because the nasopharynx harbors potential pathogens. Studies from the eastern and southern parts of Nigeria reported different types of bacterial agents cultured from the nasopharynx of children with sickle cell disease [3]. The types and number of nasopharyngeal bacteria recovered SCD patients depend on number of factors relating to the subject and administration of vaccines and antibiotics that suppress resident microflora but increase emergence of resistant strains [4]. Some investigators have shown the use of vaccines mitigate the incidence of bacterial agents such as *S. pneumoniae*, *Haemophilus influenzae* type b and *Neisseria meningitidis* which influence the carrier rate of these bacteria often implicated in acute bacterial meningitis [5]. For example in the United States, prior to the introduction of *Haemophilus influenzae* and pneumococcal vaccines, young children below the age of five years with SCD had a 13% risk of

developing bacterial sepsis with mortality rate of 30 and 10 % in patients with sepsis and meningitis [6]. A recent study of the nasopharyngeal of *S. pneumoniae* carriage among rural Gambian in West Africa sub- region, showed the nasopharynx carriage of Gambia infants by *S. pneumoniae* is rapid and highly dynamic, rising from prevalence of 1.5% at birth to 77% at 2 months, 5 months 86% and to 78% at 12 months [7]. A Brazilian study reported the nasopharynx may constitute an ecological reservoir or source of dissemination of *S. pneumoniae*, *H. influenzae*, *S. aureus* and other Gram- negative bacilli (GNB) [2]. Infants and children are the most vulnerable to opportunistic infections mainly because of their relatively immature immune system besides the elderly and the immunocompromised. We have characterized the bacterial isolates from the nasopharynx of sickle cell disease children attending the infant welfare clinic (IWC) at the Wesley Guild Hospital, Ilesa, southwestern Nigeria because the pattern of colonization, types and number of bacterial isolates from nasopharynx may differ from those reported from other regions. In addition, the study also evaluated the antibiotic resistant profile of the potential pathogenic bacterial isolates since this is an antibiotic pressurized community. We also evaluated the severity or otherwise of SCD in these patients in relation to frequency of blood transfusion and hospitalization. We believe the results obtained from our study will assist clinicians to better manage bacterial infections in children with SCD.

Methods

Study Center

Ilesa where the study took place is a semi-urban town with a population of 277,904 inhabitants in Osun State in southwestern Nigeria and the Wesley Guild Hospital is one of the outpost hospitals employed by the Obafemi Awolowo University Hospitals Complex (OAUTHC) to train medical students and other allied healthcare professionals. The hospital is located about 20 minutes' drive from Ile-Ile where Obafemi Awolowo University, the parent institution is situated.

Criteria inclusion

All participants were sickle cell disease patients that attended the infant welfare clinics of the hospital either because they were sick or had a routine checkup appointment with the attending physician. Each participant was recruited after we adequately explained the purpose of the study using each participant's native dialect. Furthermore, demographic information relating to the each participant was obtained from interviews, questionnaire responses from guardian/ parent and case files managed by the attending physicians. Ethical clearance for approval to undertake the study was given by the Ethics committee of the hospital. The age of the participants ranged from 4 months to 15 years comprising of 45 males and 39 females.

Collection of samples

Each sample was collected from each subject's pharynx by the attending physician using a sterile cotton-tipped applicator that was initially dipped into sterile normal saline and introduced into sterile thioglycolate broth. All such samples were incubated at 37°C for 24 hours for growth and further studied. Duplicate samples were prepared for anaerobes and incubated in AnaeroPack Jar 2.5 Liter, Order No.50-25, product of Mitsubishi Gas

Chemical Company Co., Inc. Japan. All samples were analyzed within 24 hours of collection.

Methods of isolation

The bacterial isolates were identified by Gram stain and growth characteristics on mannitol salt agar (Oxoid, Basingstoke, UK), blood agar, eosin-methylene blue agar, triple sugar iron agar, sulfide indole motility medium, citrate agar (Oxoid) and Analytical Profile index (API) rapid biochemical test. API kits used include API20E and API Staph (Biomerieux, France). Coagulase and catalase tests and sensitivity to Taxo A (0.04 units of bacitracin) and Taxo P (5 µg) ethylhydrocupreine hydrochloride (optochin; BD Diagnostics, Difco Laboratories, Detroit, USA) were also employed for identification. All bacterial isolates were tested for their sensitivity to commonly prescribed antibiotics using the Kirby-Bauer method. The antibiotics used were obtained from Abtek Biologicals Limited (Liverpool, UK) and included erythromycin (15 µg), gentamicin (10 µg), agumentin (30 µg), streptomycin (10 µg), tetracycline (10 µg), chloramphenicol (10 µg), nalidixic acid (30 µg), ampicillin (10 µg), nitrofurantoin (200 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), oxacillin (1 µg), kanamycin (30 µg), and *S. aureus* ATCC 25923 and *Enterobacter aerogenes* (American Type Culture Collection, Rockville, USA) were used as control organisms.

Statistical analysis

The resulting data was analysed by the descriptive analysis and t-test using SPSS version 16.0 software. Significant difference was taken as $p < 0.05$.

Results

Data relating to patients' were obtained from their records and personal files and analyzed. Table 1 shows the profile of the genotype, the frequency of blood transfusion and hospitalization of the

SCD patients. Out of the 84 SCD patients screened, 78 (92.85%) were HbSS of which 41 (52.5%) male and 37 (47.4%) female compared to 6 with HbSC of which 4 (66.7%) was male and 2 (33.3%) female. The number of blood transfusion in the 12 months prior the study was analyzed. Of the 81 patients whose records were available, 1 patient received blood transfusion 4 times; another patient had 3 blood transfusions within the period. 5 patients also received blood transfusion twice while 23 patients had only one transfusion within the period. In contrast, 51 patients did not receive blood transfusion. Regarding hospitalization in this same group was also based on the number of admis-

sion in 12 months prior to study. One patient was hospitalized 5 times, two 4 times, 5 patients were hospitalized 3 times. Furthermore, eight patients were hospitalized 2 times while 33 patients in the group were hospitalized only once. In contrast, 32 patients among the group were not hospitalized (**Table 1**).

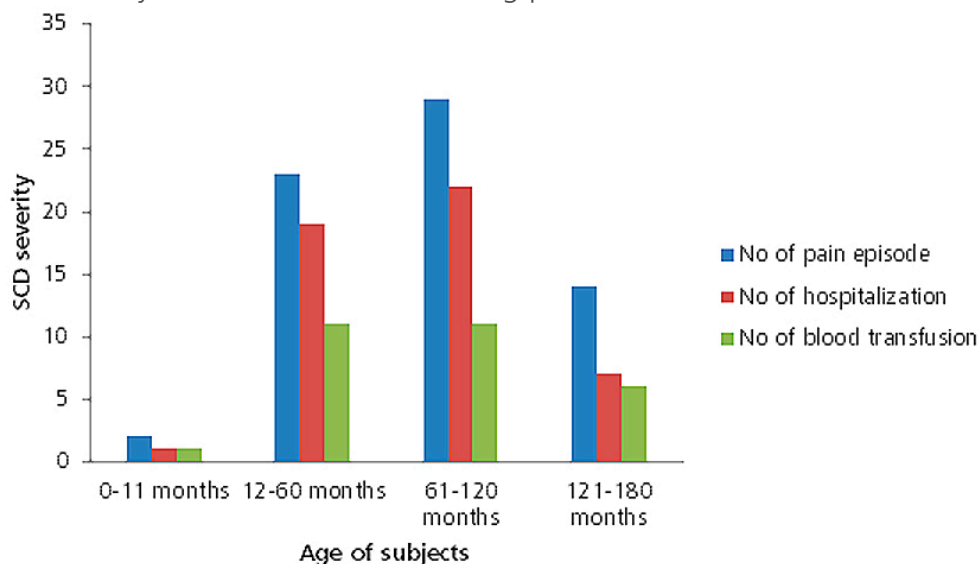
Similarly, the number of significant pain episodes in 12 months preceding the study period was assessed. SCD severity was calculated from the number of hospitalization, blood transfusion and significant pain (significant pain referred to pains necessitating hospital visits (either as outpatient or inpatient and requiring analgesics) (**Fig 1**).

Table 1. Profile of frequency of blood transfusion, number of hospitalization and Genotypes of the sickle cell patients.

Age range (months)	Total No of subject	No of times subject received blood transfusion					No of times subject was hospitalized						Subjects' Genotype			
		4	3	2	1	0	5	4	3	2	1	0	SC (M)	SC (F)	SS (M)	SS (F)
1-11	2	-	-	-	1	1	-	-	-	-	1	1	1	0	1	0
12-60	29	-	1	2	8	17	-	2	3	3	11	10	2	0	15	12
61-120	35	1	-	1	9	22	1	-	2	4	15	11	0	0	17	18
121-180	18	-	-	2	5	11	-	-	-	1	6	10	1	2	8	7
Total	84*	1	1	5	23	51	1	2	5	8	33	32	4 (66.67)	2 (33.33)	41 (52.5)	37 (47.4)

*= information on blood transfusion and number of hospitalization for three subjects was unavailable

Figure 1: Severity of sickle cell disease among patients.



Socioeconomic class (SEC) of parents was derived using the occupations and highest educational qualifications of both parents. 24 (30%) each of the SCD patients' parents were categorized in the upper and middle socioeconomic classes while 32 (40%) were classified in the low socioeconomic class.

Table 2 reflects the distribution of bacterial isolates from the nasopharynx of the 84 SCD patients. A total of 119 bacterial isolates were cultured from 84 patients averaging 1.42 bacteria/ sample. Of the 119 isolated cultured from the nasopharynx of the SCD patients, 44.53% were *Corynebacterium* spp comprising of 19 (35.84%) *Corynebacterium xerosis* 11 (20.75%) *Corynebacterium diphtheriae*, 10 (18.86) *Corynebacterium pseudodiphtheriticum*, 8 (15.09%) *Corynebacterium ulcerans*, 3 (6.66%) *Corynebacterium* spp and 2 (3.77%) *Corynebacterium jeikeium*. Other Gram positive rods cultured were *Arcanobacterium haemolyticum* 6 (5%) of the total 119 cultured, *Bacillus subtilis* was 3 (2.5%), *Actinomyces isrealii* 3 (2.5%) and *Norcadia asteroides* 1 (0.84%). Amongst the Gram positive cocci (Staphylococci) constituted 7.5% of the total bacterial isolates with CONS being 55.5% of the Staphylococci and *S. aureus* 45.5%. Streptococci constitute (2.5%) of the total bacterial isolates seen, all of which were *S. pneumoniae* isolates. The lactose fermenters constitute 9.24% of the total bacteria consisting of *Klebsiella pneumoniae* 3 (27.27%), *E. coli*, *Enterobacter aerogenes* and *Citrobacter freundii* 2 (18.18%) each and *Citrobacter diversus* and *Klebsiella oxytoca* 1 (9.09%) each. The non- lactose fermenters were 30 (25.21%) of the total bacterial isolates of which, 7 (23.33) were *Pseudomonas aeruginosa*, *Streptobacillus moniliformis* 6 (20%) each, 5 (16.66%) each of *Heamophilus influenzae* and *Sarcina spp*, *Salmonella enteritidis* 3 (10%) and *Shigella dysenteriae*, *Moraxella catarrhalis* 2 (6.66%) each.

The antibiotic resistant pattern of the four pathogens is presented in **Table 3**. Similarly **Table 4** repre-

Table 2. Distribution of bacterial isolates cultured from the nasopharynx of SCD subjects.

	Bacterial isolates	Total no of bacterial cultured	Total No (%)
Bacilli			
Gram Positive Rods	<i>Corynebacterium xerosis</i>	19	15.96
	<i>Corynebacterium diphtheriae</i>	11	9.24
	<i>Corynebacterium pseudodiphtheriticum</i>	10	8.4
	<i>Corynebacterium ulcerans</i>	8	6.7
	<i>Arcanobacterium haemolyticum</i>	6	5.0
	<i>Corynebacterium spp</i>	3	2.5
	<i>Bacillus subtilis</i>	3	2.5
	<i>Actinomyces isrealii</i>	3	2.5
	<i>Corynebacterium jeikeium</i>	2	1.7
	<i>Norcadia asteroides</i>	1	0.84
Gram Positive Cocci			
	CONS (coagulase negative Staphylococci)	5	4.2
	<i>Staphylococci aureus</i>	4	3.4
	<i>Streptococcus pneumoniae</i>	3	2.5
Gram Negative Rods			
Lactose fermenters	<i>Klebsiella pneumoniae</i>	3	2.5
	<i>Escherichia. coli</i>	2	1.7
	<i>Enterobacter aerogenes</i>	2	1.7
	<i>Citrobacter freundii</i>	2	1.7
	<i>Citrobacter diversus</i>	1	0.84
	<i>Klebsiella oxytoca</i>	1	0.84
Non lactose fermenters	<i>Pseudomonas aeruginosa</i>	7	5.9
	<i>Streptobacillus moniliformis</i>	6	5.0
	<i>Heamophilus influenzae</i>	5	4.2
	<i>Sarcina spp</i>	5	4.2
	<i>Salmonella enteritidis</i>	3	2.5
	<i>Shigella dysenteriae</i>	2	1.7
	<i>Moraxella catarrhalis</i>	2	1.7
	Total		119

Table 3. Antibiotic resistant pattern of potential pathogenic bacterial isolates cultured from the nasopharynx of SCD subjects at the Wesley Guild Hospital, Ilesha

Bacterial Isolates tested	Total No tested	No (%) tested	B lactams			Cephalosporins	Macrolides	Tetracycline	Aminoglycosides			Chloramphenicol	Quinolones	Fluoroquinolones	Nitrofurantoin
			AUG	AMP	OXA				CRO	ERY	TET				
<i>Haemophilus influenzae</i>	5	5	3 (60)	4 (80)	4 (80)	4 (80)	3 (60)	5 (100)	2 (40)	0	0	5 (100)	5 (100)	3 (60)	3 (60)
<i>Staphylococcus aureus</i>	4	4	1 (25)	4 (100)	0	0	4 (100)	1 (25)	0	1 (25)	0	0	4 (100)	0	0
<i>Streptococcus pneumoniae</i>	3	3	3 (100)	3 (100)	3 (100)	3 (100)	0	1 (33.33)	2 (66.67)	0	0	3 (100)	3 (100)	0	0
<i>Moraxella catarrhalis</i>	2	2	1 (50)	2 (100)	2 (100)	2 (100)	1 (50)	0	0	0	0	2 (100)	2 (100)	0	0

Keys to abbreviations: TET = Tetracycline STREP = Streptomycin; AMP = Ampicillin; GEN = Gentamycin; AUG = Augmentin; CRO = Ceftriaxone; OXA = Oxacillin; CHL = Chloramphenicol; ERY = Erythromycin; KAN = Kanamycin; NIT = Nitrofurantoin; CIP = Ciprofloxacin; NAL = Nalidixic acid; Value in parenthesis=Number in %

Table 4. Multiple Antibiotic Resistance Index of the predominant pathogenic bacterial isolates cultured from the nasopharynx of SCD subjects at Wesley Guild Hospital, Ilesha.

Class of Antibiotic	Antibiotics used	Total no. of isolates tested	Total no. (%) resistant	Total no. (%) susceptible	MAR index
β-lactams	<i>Ampicillin</i>	14	13	1	0.92
	<i>Augmentin</i>	14	8	6	0.57
	<i>Oxacillin</i>	14	9	5	0.64
Cephalosporins	<i>Ceftriaxone</i>	14	9	5	0.64
Tetracyclines	<i>Tetracycline</i>	14	7	7	0.50
Macrolides	<i>Erythromycin</i>	14	8	6	0.57
Aminoglycosides	<i>Streptomycin</i>	14	4	10	0.28
	<i>Gentamycin</i>	14	1	13	0.07
	<i>Kanamycin</i>	14	0	14	0
Chloramphenicol	<i>Chloramphenicol</i>	14	10	4	0.71
Quinolones	<i>Nalidixic acid</i>	14	14	0	1.0
Fluoroquinolones	<i>Ciprofloxacin</i>	14	3	11	0.21
Nitrofurans	<i>Nitrofurantoin</i>	14	3	11	0.21

sents the multiple antibiotic resistances of the four pathogens using MAR index.

Discussion and conclusion

The sickle gene confers an increased susceptibility to infection, especially to certain bacterial pathogens, and at the same time infection provokes a cascade of SCD-specific pathophysiological changes. Africa bears the highest burden of sickle cell disease on the globe. Our study characterized the nasopharyngeal bacterial isolates cultured from 84 Sickle cell disease patients at the infant welfare clinic of the Wesley Guild Hospital, of which 78 (92.85%) were HbSS of this 41 (52.56%) were males and 37 (47.44%) females. In addition, 6 (7.14%) were HbSC; 4 (66.66%) males and 2 (33.33%) females. Despite the fact that over 700 structural haemoglobin (Hb) variants have been identified worldwide, only (Hb S, Hb C) constitutes high frequencies in Africa [8]. Altogether, 119 isolates

were cultured from the nasopharynx of the SCD patients, consisting of 78 (65.54%) Gram positive bacteria comprising 66 (55.46%) Gram positive rods and 12 (10.08%) Gram positive cocci dominated and Gram negative isolates was (34.45%) of total bacterial recovered from SCD patients. The predominant Gram positive organism was *Corynebacterium* spp comprising 44.53% of the Gram positive rods of which *Corynebacterium xerosis* predominated. The Gram positive cocci were 5 (4.20%) CONS and *S. aureus* 4 (3.36%) and 3 (2.52%) *S. pneumoniae* underscoring a low rate of nasopharyngeal colonization with these organisms in this center which may be attributable with the prophylactic use of penicillin in this center. Despite the high nasopharyngeal carriage which often is a determinant for invasive pneumococci in some regions [9], it is puzzling to note high carriage rates with low rates of invasive pneumococcal disease in sub-Saharan Africa [10, 11]. According to Kizito *et al.*, [11] a significantly low rate of pneumococ-

cal bacteremia in Ugandan children with sickle cell disease (6%, 3/47) [12], and other studies have indicated low pneumococcal bacteremia in Nigerian children [13, 14]. However, in Zambia low rate of pneumococci frequency was attributable to strict antibiotic policy of the government that reduced access to sale of over the counter drugs. Our data showed widespread multiple antibiotic resistance among the four pathogens we tested. Our study shows all the 5 *H. influenzae* isolates tested were resistant to tetracycline, chloramphenicol and nalidixic acid. 4 isolates were resistant to ampicillin, oxacillin and third generation cephalosporin-ceftriaxone while 3 isolates were resistant to augmentin, erythromycin, ciprofloxacin and nitrofurantoin. In addition, two of *H. influenzae* isolates were also resistant to streptomycin but none was resistant to gentamycin and kanamycin. Similarly, of the four *S. aureus* isolates cultured, all were resistant to ampicillin, erythromycin and nalidixic acid and 1 isolate was resistant to augmentin, tetracycline and gentamycin but none was resistant to oxacillin, ceftriaxone, streptomycin, kanamycin, chloramphenicol, ciprofloxacin and nitrofurantoin. Furthermore, our results reveal among the three *S. pneumoniae* isolates cultured from the nasopharynx, all were resistant to the beta lactams antibiotics, augmentin, ampicillin, oxacillin and ceftriaxone. Two of the *S. pneumoniae* isolates were resistant to streptomycin and one isolate to tetracycline, chloramphenicol and nalidixic acid. In contrast, none of the isolates was resistant to erythromycin, gentamycin, kanamycin, ciprofloxacin and nitrofurantoin. Finally, among the *Moraxella catarrhalis* isolates tested, all were resistant to ampicillin, oxacillin, ceftriaxone, chloramphenicol, nalidixic acid. However, 1 isolate was resistant to augmentin, erythromycin but all were sensitive to the other antibiotic used (Table 3). Because of susceptibility to severe pneumococcal infection, children with sickle cell disease (SCD) routinely receive penicillin prophylaxis which unintentionally

increased the rates of penicillin resistance reported throughout the world, this practice has led to emergence of penicillin resistant *S. pneumoniae* (PRSA) untamable to treatment with β -lactams in particular and also a variety of different antibiotics.

The predominance of *Corynebacterium* spp seen in our study among these SCD patients may also be related to indigenous microflora which represents commensals of the nasopharynx that limit the growth of pathogenic organisms by phenomenon of microbial antagonism. Furthermore, Gram negative organisms accounted for 34.46% of the nasopharyngeal isolates comprising of 11 (26.82%) lactose fermenters and 30 (73.17%) non lactose fermenters. Colonization with Gram negative organisms specifically with lactose fermenters- *E. coli*, *Klebsiella*, *Citrobacter* and *Enterobacter* spp is of concern but may be attributable to patients' personal hygiene. It is interesting to note that 40% of the population studied comes from low socioeconomic group (15) who live in overcrowded living conditions and lack portable drinking water are therefore at higher risk of parasitic infections such as malaria and diarrhoea. The low rate of nasopharyngeal colonization in children at this center with *Haemophilus influenzae* 5 (4.2%) and *S. pneumoniae*, may be attributed to administration of vaccines early in life in this environment.

The multiple antibiotic resistance (MAR) seen in our study was widespread among the four predominant pathogens. The MAR indices for the beta-lactams for example, comprising of ampicillin, augmentin, oxacillin and ceftriaxone were 0.92, 0.57, 0.64 and 0.64 respectively. For tetracycline, the MAR value was 0.50; and for erythromycin 0.57. Regarding aminoglycosides comprising of streptomycin, gentamycin and kanamycin their MAR indices were 0.28, 0.07 and 0.00 respectively. For chloramphenicol the MAR index was 0.71, while for nalidixic acid it was 1.00. When we analyzed ciprofloxacin and nitrofurantoin, MAR value was 0.21 for each antibiotic. These results

suggest widespread multiple antibiotic resistance among organisms cultured from SCD patients. Our study showed an unexpected high frequency of nasopharyngeal colonization rarely seen with indigenous microflora specifically *Corynebacterium* spp at this center. This finding suggests routine use of prophylactic penicillin may have suppressed *S. pneumoniae* nasopharyngeal colonization among the subjects. It also suggests routine vaccination of infants and children in the center may be assisting in reducing the frequency of colonization by *S. pneumoniae* and *Haemophilus influenzae* among subjects. Our data show beta- lactam antibiotics are virtually ineffective against the organisms cultured from these SCD patients compared with aminoglycosides, quinolones and nitrofurantoin. This observation is of epidemiological significance in the event of an epidemic in this center. The widespread resistance recorded for the majority of nasopharyngeal isolates recorded in our study requires urgent and necessity of instituting effective antibiotic policy in this center to reduce the frequency of drug- resistant in this population.

Studies have shown SCD not only originates from abnormality of RBC but also is a multisystem disorder, affecting virtually every organ of the human body. These conditions include haemolysis, many haematological complications, vasoocclusion, infection and organ dysfunction (8). Our study reveals 49 (60.49%) of the SCD patients were hospitalized at least once. Thirty (61.22%) of these patients also received blood transfusion at least once. SCD patient suffers chronic haemolytic anaemia which allows the patient to carry on normal activities at steady state hemoglobin with narrow reserve capacity to accommodate strenuous physical activities [16, 17, 18, 19]. The role of the blood transfusion in the SCD patient is meant at increasing the level of hemoglobin, improve oxygen delivery and reduce proportion of sickle RBCs in circulation [8]. In addition, infection caused by *H. influenzae* and *S. pneumoniae* is a major cause of

concern for children with SCD with haemoglobin SS; HbSS and HbSC as these children are at risk of bacteremia and chronic anemia that lead to early loss of splenic function [6]. Our results, however suggest despite their genotypes, these children had low rate of colonization with *H. influenzae* and *S. pneumoniae* at this center which is interesting and may lead to further reduction in infection with these organisms. Furthermore, the preponderance of indigenous microflora in nasopharynx of these children by organisms such as *Corynebacterium* spp most of which are commensals, are in competition with pathogens because of microbial antagonism, an aspect of innate immunity will reduce the colonization with pathogens. Besides, administration of both pneumococci and influenzae vaccines in booster doses may also lower the rate of nasopharyngeal colonization [20]. In conclusion, Gram positive bacterial isolates predominated in this study (65.45%) compared to (34.55%) of Gram negative bacteria. However *Corynebacterium* spp predominated. Low rate of nasopharyngeal colonization with *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Staphylococcus aureus* strains was recorded. The study also recorded widespread multiple drug resistance among most isolates colonizing the nasopharynx of SCD in this center which is worrisome suggesting for urgent institution of effective antibiotic policy to stem the tide of multi-drug resistant organisms in this center.

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