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Resistance Phenotypes of Bacterial Strains Responsible for Urinary Infections and Bacteraemia among Febrile Children with Sickle Cell Anaemia in Mali

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Abstract

The broad-spectrum empiric antibiotic therapy recommended for sickle-cell fever can help to develop bacterial resistance. The purpose of this study is to explore and describe resistance phenotypes of isolated bacterial strains of urinary infections and bacteraemia among febrile children with sickle-cell anaemia. From April 2014 to March 2016, we collected 25 bacterial strains from febrile sickle-cell patients aged 6 months to 15 years. The strain was identified by a conventional standard procedure which includes morphological study, culture and biochemical characteristics of the strain followed by an automated method. Twenty-five (25) bacterial strains were isolated in 25 sickle cell children, comprising 17 SS, 3 SC and 5 S-thalassemia strains. These bacterial strains were isolated in urine (23/25 or 92%) and blood cultures. *E. coli, Klebsiella pneumoniae, Enterobacter spp., Serratia ficaria, Raoultella ornithinolytica, Pantoea spp.* and *Salmonella spp* were isolated from urine and blood cultures respectively. All these isolated strains were *Enterobacteria*, with 56% (14/25) *E. coli*, 16% (4/25) *Klebsiella pneumoniae*, 8% (2/25) *Salmonella group*, 8% (2/25) *Enterobacter spp*, 4% (1/25) *Serratia ficaria*, 4% (1/25) *Raoultella ornithinolytica*, and 4% (1/25) *Pantoea spp*. More than half of the strains were isolated in female patients (19/25). The antibiogram revealed ESBL-producing strains (28% of 7/25) and Cephalosporinasis (8% or 2/25). The isolated strains showed 4% (1/25) resistance to all the aminoglycosides tested, whereas all the strains isolated from the blood cultures showed a wild phenotype. Resistance to quinolones associated with alteration of gyrA and parC genes was 4% (1/25) for all strains isolated from the blood and urine cultures. The resistance phenotypes of bacterial strains found in our study are variable. This study suggests that we should give more priority to other empirical antibiotherapies that use the molecules of the aminoglycosides and quinolones family for sickle cell children in our context than to those recommended elsewhere.

Keywords: Resistance; Phenotypes; Urinary infection; Bacteremia; Sickle cell children; Mali

Introduction

Bacteremia and urinary tract infections are some of the causes of morbidity and mortality among children [1]. Relevant literature reveals that bacterial species of the enterobacteria family and others are among the microorganisms involved in these infections [1,2]. They are the second leading cause of serious infections after negative Gram cocci. These bacteria have been reported by several authors in septicemia, urinary tract infections and osteomyelitis among children with sickle cell disease [3-6]. Enterobacteria other than salmonellae appear to be found with abnormally high frequency in sickle cell patients during urinary and bone infections [7] and septicaemia [8]. This high susceptibility of sickle-cell anaemia to infections is well known; it is related to functional asplenia, which is the consequence of repeated splenic infarcts [9-11]. When not treated, these infections can lead to serious complications with life-threatening risks for sickle-cell patients. In 1986, Gaston et al. in a principal study demonstrated that penicillin V administered systematically to homozygous SS sickle cell patients could significantly reduce mortality associated with these infectious complications. Since then, several health care protocols and international recommendations have advocated the systematic prescription of broad-spectrum antibiotics for febrile sickle cell patients [10,12-15]. However, this probalistic prescription of antibiotics within such a frequent context for this vulnerable population helps to develop antimicrobial resistance, resulting in prolonged hospitalization, excessive financial costs and exposure of patients to various complications. To the best of our knowledge, no studies on the assessment of antimicrobial resistance have so far been conducted among sickle cell patients in Mali. Our study attempts to determine resistance phenotypes of bacterial strains isolated during fever (temperature ≥ 38°C) among sickle cell children, and streamline their therapeutic management.

Results

Over a period of 24 months, 25 bacterial strains were isolated from 25 sickle cell children aged 6 months to 15 years. The average age was

5.20 ± 2.9, and the sex ratio was 0.31. Antibiotics taken by all the children before this study were documented. The average hospitalization period was 2.36 ± 1.9 in our study population. Among these children, there were 17 (68%) SS homozygotes, 3 (12%) SC double heterozygotes, and 5 (20%) Sβ-thalassemics. These bacterial strains were isolated from the urine cultures (92% of 23/25) and the blood cultures (8% or 2/25) (Table 1).

Characteristics	Population considered
Sex (n=25)	
F	19
M	6
Age of the population considered (n=25)	
Average age ±DS	5.2±2.9
Sickle cell phenotypes (n=25)	
SS	17
SC	3
S/β	5

Table 1: Characteristics of the sickle cell children considered; DS: Deviation from Standard.

The strain was identified by a conventional standard procedure which includes morphological study, culture and biochemical characteristics of the strain followed by an automated method. *E. coli, Klebsiella pneumoniae, Enterobacter spp., Serratia ficaria, Raoultella*

ornithinolytica, Pantoea spp. and *Salmonella spp* were isolated from the urine and blood cultures respectively. All these isolated strains were *Enterobacteria*, with 56% (14/25) *E. coli*, 16% (4/25) *Klebsiella pneumoniae*, 8% (2/25) *Salmonella group*, 8% (2/25) *Enterobacter spp*, 4% (1/25) *Serratia ficaria*, 4% (1/25) *Raoultella ornithinolytica*, and 4% (1/25) *Pantoea spp* (Table 2).

Origin of Strains			
Bacteria Strains	Blood (Percentage 100 xn/2)	Urine (Percentage 100xn/23)	Number of Strains (n)
E. coli	--	14 (60.87)	14
K. pneumoniae	--	04 (17.39)	4
Salmonella spp	02(100)	--	2
Enterobacter spp	--	02 (8.69)	2
Serratia ficaria	--	01 (4.35)	1
Raoultella ornithinolytica	--	01 (4.35)	1
Pantoea spp	--	01 (4.35)	1
Total	02(100)	23 (100)	25

Table 2: Distribution of bacteria strains isolated from the various samples.

We also noted cross-resistance phenotypes in the beta-lactam family (Table 3).

Phenotypes resistant to betalactamines									
Bacteria strains isolated	Wild 4%	BLSE 12%	PHN 36%	CHN 4%	TRI 4%	BLSE+CHN 16%	BLSE +PHN 4%	PeniQ+CHN +IRT 4%	PeniQ+BLSE+ CHN 16%
E. coli (n=14)	1	2	1	1	--	3	1	1	4
K. pneumoniae (n=4)	--	2	1	--	1	--	--	--	--
Salmonella spp (n=2)	--	2	--	--	--	--	--	--	--
Enterobacter spp (n=2)	--	1	--	--	--	1	--	--	--
Serratia ficaria (n=1)	--	1	--	--	--	--	--	--	--
Raoultella ornithinolytica (n=1)	--	--	1	--	--	--	--	--	--
Pantoea spp (n=1)	--	1	--	--	--	--	--	--	--

Table 3: Distribution of phenotypes resistant to betalactamines of the isolated bacteria strains; PBN: Low Level Penicillinase; PHN: High Level Penicillinase; TRI: Resistant to inhibitors; CHN: High Level Cephalosporinase; BLSE: Broad Spectrum Beta-lactamase; PeniQ: Acquired Penicillinase.

Methodology

The study was conducted from April 2014 to April 2016, covering a period of 24 months. It was a cross-sectional descriptive and prospective study. Bacterial strains were isolated from urine and blood cultures from children with major sickle cell syndromes (SS, SC, S/ß-thalassemia) followed up at "Centre de Recherche et de Lutte contre la Drépanocytose" (CRLD), who were consulted and/or hospitalized for

fever. The bacteriological study of these strains was conducted at the Medical Biology Laboratory of the CRLD for the urine cultures, and at the Biology Laboratory of the "Centre d'Infectiologie Charles Mérieux" (CICM) of Bamako for the blood cultures. The identification of bacterial strains and the study on antibiotic susceptibility were conducted with the VITEK® 2 Compact system using antibiogram (AST-N222 and AST-N233) and identification (GN) maps. The antibiotics tested included betalactamins, aminoglycosides, and

quinolones. Resistance phenotypes were determined automatically using the VITEK® 2 Compact system; the quality control of the antibiotics tested was conducted using the *E. coli* ATCC 25922 baseline strain (FDA Strain Seattle 1946).

More than half of the strains were isolated among female patients (76% or 19/25) of the 6 months-5 years age group. The antibiogram revealed Betalactamase (ESBL)-producing strains (36% or 9/25) and Cephalosporinasis (8% or 2/25). The isolated strains showed 4% (1/25) resistance to all the aminoside tested, whereas all the strains isolated from the blood cultures showed a wild phenotype with aminosides (Table 4).

Bacteria strains isolated	Aminoside Phenotypes		Quinolone Phenotypes		
	Wild 96%	KTG 4%	Wild 88%	2GyrAParC 4%	Partial Resistance 8%
E. coli	14	--	14	--	--
K. pneumoniae	4	--	4	--	--
Salmonella spp	2	--	--	--	2
Enterobacter spp	2	--	2	--	--
Serratia ficaria	--	1	--	1	--
Raoultella ornithinolytica	1	--	1	--	--
Pantoea spp	1	--	1	--	--

Table 4: Distribution of phenotypes resistant to aminosides and quinolones of the isolated bacteria strains.

K: Kanamycine; T: Tobramycine; G: Gentamicine

2GyAParC: 2-mutation Phenotype of gyrA and parC genes.

Resistance to quinolones associated with alteration of gyrA and parC genes was 4% (1/25) for all strains isolated from the blood and urine cultures. Resistance to quinolones associated with alteration of gyrA and parC genes was 4% (1/25), and partial resistance was 8% (2/25) for all the strains isolated in our study (Table 4).

Discussion

This study focused on bacterial strains isolated from urinary cultures and during bacteremia among febrile sickle-cell children in Mali. It shows a high number of SS homozygotes among our study population, which reflects what obtains among the sickle cell patient population followed up at the CRLD. The predominance of females in our study population could be due to the shortness of their urethra, proximity to the anal and vaginal openings, and probably inadequate hygiene practices [16,17]. Patients aged 6 months to 5 years accounted for 56.3% of our study population, and 43.7% for those aged 6 to 15 years. The predominance of the 6 months to 5 years age group could be due to immunitary immaturity, which makes the immune defense system more or less ineffective during this period of life among sickle cell patients [10,11]. These results are higher than those reported in the relevant literature [18], with a rate of 37.1% for the 0 to 5 years age group. *E. coli* is by far the most common enterobacterium found in urinary tract infections in our study population, presumably because

of its ability to adhere to cells, followed by *Klebseilla pneumoniae*. Our results are comparable to those reported in Senegal among the general public [19]. However, these results are similar to those obtained in 2011 and in 2014, in a case-control study in North-Eastern Nigeria in Maiduguri, to determine the prevalence of bacteriuria and bacterial isolates in the urine of febrile children with major sickle cell syndrome, as well as a study conducted in India which assessed the role of serum procalcitonin as biomarker of bacterial infection among sickle cell patients in vaso-occlusive crisis [4,20]. Like in other studies on sickle cell patients, *Salmonella* is the second most commonly isolated bacterial microorganism during bacteremia in a sickle cell patient population in Kenya, and the third in England and the United States, with proportions of 20% to 50% of bacteraemia among these patients. Our results are similar to those reported by these authors [21-25]. On the other hand, it occupies the first position in a study conducted in Libreville [3]. The study on susceptibility to antibiotics showed significant resistance of enterobacterial strains to all the antibiotics tested. The resistancemainly concerned betalactamins, cephalosporins, aminoside and quinolones. They are acquired and are believed to stem from a probabilistic antibiotic therapy recommended by most of the protocols that advocate the systematic prescription of broad-spectrum antibiotics [10,12-15], as well as from uncontrolled and abusive consumption of these molecules, aggravated by cases of fever among sickle cell patients. The antibiotic susceptibility study revealed a high level of ESBL-producing enterobacterial strains of 28.13% for the beta-lactam resistance phenotype. This could strongly contribute to the emergence of resistance among the general public [26]. The steady increase in the frequency of ESBL-producing strains is related to the emergence of strains that are resistant to C1G, cefalotine (60%) and cefotaxime (70%) in hospitals [27]. In our study population, this would probably be related to international recommendations advocating the systematic prescription of broad-spectrum antibiotics in cases of fever in general, and for sickle cell children, in particular. We identified strains in our study with cross-resistance in the beta-lactam family. The resistance was 34% for all strains of enterobacteria isolated during urinary tract infections and bacteremia in our study population. The isolated strains retained quite acceptable sensitivity to aminoglycosides with 4% KTG resistance phenotype. This low level of KTG resistance phenotype could be due to the emergence of 16S RNA methyltransferase enzyme which confers a high level of resistance to all the aminoside used in common practice. Our results are similar to those reported in 2003 in a study conducted on the susceptibility of enterobacteriaceae to antibiotics in Northern Liban on over a four-year period among the general public [28]. In a study that focused only on *E. coli*, an acceptable sensitivity of enterobacteria to antibiotics was reported [29]. For the quinolones tested, approximately 4% of strains exhibited high level resistance associated with alteration of gyrA and parC genes. Such emergence of quinolone-resistant strains could stem from the implementation of recommendations and consensus conference on primary prescription of quinolones as replacement for amoxicillin [30]. Wild phenotypes were more frequently identified for aminoside with rates of 96% and 88% for quinolones, and only 4% for betalactamins.

Conclusion

Resistance to antibiotics is a constantly emerging phenomenon. Resistance phenotypes of bacterial strains were frequently found in urinary tract infections and bacteremia among sickle cell children in Mali. This study suggests the use of other antibiotics, including

macrolides and quinolones, during infections among sickle cell children in Mali.

Acknowledgment

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