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Full Length Research Paper

Assessment of the prevalence of extended-spectrum β-lactamase producing Gram-negative bacilli at the Charles De Gaulle Paediatric University Hospital (CDG-PUH), Ouagadougou, Burkina Faso

Mahamoudou Sanou[1,2,5*], Absétou Ky/Ba[1], Pricille Coulibali[1], Marius Nagalo[4], Abdoul Salam Ouédraogo[3], Mamadou Tamboura[2], Dinanibé Kambiré[2], Cyrille Bisseye[4], Fidèle Bakiono[6], Jacques Simporé[4] and Ramata Ouédraogo[1,2]

[1]Training and Research Unit in Health Sciences (UFR-SDS), University of Ouagadougou, Ouagadougou, Burkina Faso.
[2]Charles-de-Gaulle Paediatric University Hospital (CHUP-CDG), Ouagadougou, Burkina Faso.
[3]Souro Sanou University Hospital (CHU-SS), Bobo-Dioulasso, Burkina Faso.
[4]Pietro Annigoni biomolecular Research Centre (CERBA)/LABIOGENE, University of Ouagadougou, Burkina Faso.
[5]General Lamizana Camp Medical Centre, Burkina Faso.
[6]Armed Forces Health Service, Ouagadougou, Burkina Faso.

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Bacterial resistance to antibiotics is a serious concern in developing countries due to precarious hygiene conditions, inappropriate prescription as well as self-medication and free sale of antibiotics. This study was aimed to assess the prevalence of strains producing extended-spectrum beta-lactamase in the Gram negative bacteria isolated in the laboratory. The study was conducted in the Charles De Gaulle Paediatric University Hospital (Ouagadougou) and the Arnaud de Villeneuve Regional University Hospital (Montpellier). Out of the 889 pathological substances (pus, urine and blood) analysed, 175 germs were isolated among which 110 were Gram negative bacteria (62.8%). 48.2% of the Gram negative bacteria were positive to extended-spectrum beta-lactamase. Among the 110 Gram negative bacteria isolated, 101 were Enterobacteria and 9 other Gram negative bacteria. No extended-spectrum beta-lactamase was found in the other Gram negative bacteria and 52.5% of Enterobacteria were extended-spectrum beta-lactamase positive. As for the extended-spectrum beta-lactamase phenotype, 60.4% were *Escherichia coli* and 32% were *Klebsiella pneumoniae*. 50.9% of extended-spectrum beta-lactamase came from pus and 41.5% from urine. In addition, 64.6% of hospitalised patients had an extended-spectrum beta-lactamase phenotype compared to 24.5% for out-patients. The results show the importance of the phenomenon and should help to better take care of this scourge because antibiotics despite everything, always save millions of lives.

Key words: Extended-spectrum beta-lactamase (ESBL), enterobacteria, gram-negative bacilli, inpatients, out-patients.

INTRODUCTION

As a global concern, bacterial resistance to antibiotics is becoming a serious worry in developing countries due to

precarious hygiene conditions, inappropriate prescription as well as self-medication and free sale of antibiotics. Gram Negative Bacilli (GNB) are frequently implicated in human infections and Enterobacteria are the most commonly isolated bacterial species in the laboratory.

The secretion of extended-spectrum beta-lactamase (ESBL), a common mechanism of bacterial resistance to antibiotics, is becoming a threat to public health. Indeed, the ESBL bacteria are more and more resistant to all beta-lactams (except cephamycins and Carbapenems). In addition, the plasmids supports have bigger size and have genes resistant to other antibiotics causing Multidrug resistant (MDR) bacteria emergence (Boyd et al., 2004).

Perhaps in developed countries, the extended surveillance and the particular care provided to ESBL carriers have reduced the antimicrobial resistance; however, in under-developed countries, the unlimited accessibility to beta-lactams and the abusive use of those molecules contribute to expand the antibiotics resistance phenomenon (Livermore, 1995; Sangare et al., 2015; Storberg, 2014). In Burkina, this phenomenon is particularly marked in recent years with the free dispensing of ceftriaxone generic drug. From 15% in 2007 (Ouedraogo et al., 2011), the drug resistance went to 33% in 2013 (Ouedraogo et al., 2016).

The study aims to assess the scope of the phenomenon of bacterial resistance to antibiotics in the developing countries due to precarious hygiene conditions, inappropriate prescription as well as self-medication and free sale of antibiotics. The primary objective was to evaluate the extent of the phenomenon and identify ESBL-producing strains among the GNB isolated in the Charles-de-Gaulle Paediatric University Hospital (CDG-PUH) laboratory. Such study should help for emergency measures to fight it.

MATERIALS AND METHODS

The study was conducted at the Charles De Gaulle Paediatric University Hospital (CDG-PUH) in Ouagadougou, Burkina Faso and the Arnaud de Villeneuve Regional University Hospital (ADV-RUH) in Montpellier, France. It is a prospective cross-sectional descriptive study covering from 1st May to 12 August 2014.

The study population consisted of all patients who performed a bacteriological examination in the CDG-PUH laboratory. Included in this study were all patients in whom bacteriological analysis of the specimen (pus, urine and blood) resulted in isolation and identification of a GNB during the study period. Patients' or accompanying relatives' consent was received before the beginning of this research. Samples containing GNB were seeded on appropriate culture media for isolation. Non-selective media (CLED agar) and / or selective (Hektöen agar, EMB) were used according to the pathological product and incubated in an oven at 37°C for 24 to 48 h.

The GNB obtained were reisolated on Muller Hinton medium to obtain pure cultures for preservation. The pure colonies were scraped with a seed and discharged into a cryotube containing a preservation medium (the "Protect" medium) and stored at -20°C until shipped to Montpellier in France, in Arnaud de Villeneuve Regional University Hospital (ADV-RUH). The Matrix-Assisted Laser Desorption/Ionisation-Time-of-Flight (MALDI-TOF) (automated bacteriology), the principle of which is based on mass spectrometry, was used to identify and analyze the bacterial protein. The antibiotic susceptibility testing was carried out through the dissemination technique on Mueller Hinton Agar plate according to antimicrobial committee of France microbiology society (CA-SFM) and the complete automated interpretation was done with SIRScan micro™.

Statistical analyses were performed using Epi-Info 7.2. Groups were compared using Pearson's or Yates χ^2 test, when needed. For all analyses, a p-value lower than 0.05 was considered as significant.

RESULTS

Socio-demographic characteristics of sample

In this study, 106 patients were included. Male patients were the majority (54.7%), giving a sex ratio of 1:2. The age group 1 to 30 months was the most important one. Out of the 106 patients, 60.4% were in-patients compared to 39.6% out-patients. Most of the hospitalised patients were from the surgical ward and represented 59.4%. Sixty six patients took antibiotics before the bacteriological test and represented 62.3% (Table 1).

Bacteriological results

Out of 889 pathological substances analysed, 175 germs have been isolated among which 110 (62.8%) were Gram negative bacteria. These 110 Gram negative bacteria were from 106 samples among which 102 were monomicrobials and 4 bimicrobials. A predominance of *E. coli* (51.8%) was observed, followed by *K. pneumoniae* with 26 germs representing 23.6%. *P. aeruginosa* came in third position with 7 germs representing 6.4%. Most of the microbes were found in urine and pus with a distribution of 55 cases (50%) and 46 cases (41.8%), respectively.

Out of the 110 germs isolated, 53 (48.2%) produced an extended spectrum of β-Lactamase. We also noted 101 enterobacteria and 9 other Gram negative bacteria. No production of extended spectrum of β-lactamase was

*Corresponding author. E-mail: mahamoudsanou@hotmail.com

Table 1. Socio-demographic characteristics.

Age (months)	Number (n)	Percentage (%)
< 1	09	08
1-30	39	37
31-60	07	07
61-120	24	23
121-180	14	13
> 180	13	12
Total	106	100
Sex		
Male	58	55
Female	48	45
Total	106	100
Status		
In-patients	64	60
Out-patients	42	40
Total	106	100
Services		
Surgery	38	59
Older children	07	11
Infectious diseases	05	08
Neonatology	05	08
Infant	04	06
Others	05	08
Total	64	100

observed in the other Gram negative bacteria; the 53 ESBL producing germs were enterobacteria and represented 52.5%. As for the ESBL Phenotype, according to the species/gender, 60.4% of ESBL were *E coli* and 32% were *K pneumoniae,* 5.7% were *Enterobacter cloacae* and 1.9% were *Providencia stuartii* (Table 2). Most ESBL were found with *Escherichia coli* and *Klebsiella pneumonia* both representing 92.4% cases respectively equivalent to 60.4 and 32% of the cases.

In relation to each species, strong prevalence of ESBL has been observed: 65.4% *Klebsiella pneumoniae* had the ESBL phenotype; thereafter, *Escherichia coli* with 56.1% cases; *Enterobacter cloacae* and *Providencia stuartii* were in the third position with each 50% of cases (Table 2). The ESBL production frequency was 24.5% in out-patients and 64.6% in hospitalised ones. The value of $p < 0.05$ implies that there is a significant difference between the ESBL production within in-patients compared to out-patients. There are about 6 times (Odds Ratio = 5.64) more ESBL production risks in hospitalised patients than in non-resident ones (Table 3).

According to previous antibiotherapy, 79.2% ESBL production germs came from patients who had taken antibiotics in the last 3 months before the bacteriological test. ESBL producing bacteria were found in patients aged 1 to 30 months (32.1%) followed by patients aged 61 to 120 months (26.4 %) (Table 4).

Out of the 110 isolates, 43 (39.1%) manifested a resistance phenotype to β-lactams different from the ESBL phenotype. However, no carbapenemase phenotype has was observed (Figure 1).

Resistance phenotypes of isolates to aminoglycosides

Out of the 110 isolates, 60 (54.5%) presented at least one of the resistance phenotypes to aminoglycoside. *Klebsiella pneumoniae* manifested the highest resistance to aminoglycoside with 69.2% cases.

The GTNt phenotype (gentamicin, tobramycin, and netilmicin resistance) was mostly represented. No resistance phenotype to aminoglycoside was found in *Pseudomonas aeruginosa.*

Table 2. Distribution of ESBL producing germs according to bacterial species identified.

Identified specie	ESBL		
	Number (n)	Percentage (%) (related to ESBL total : n=53)	Percentage (%) (related to each species)
K. pneumoniae	17	32.0	65.4
E. coli	32	60.4	56.1
Enterobacter cloacae	3	5.7	50
Providencia stuartii	1	1.9	50
Total	53	100.0	-

Table 3. Distribution of ESBL producing germs according to status at hospital.

Status	ESBL		ESBL+		P
	N	Percentage (%)	N	Percentage (%)	
Out-patients	34	75.5	11	24.5	< 0.001
In-patients	23	35.4	42	64.6	

Yates' Chi square corrected = 15.6 $p = 1.7\ 10^{-5}$. Odds Ratio = 5.64.

Resistance of isolates to quinolones

Out of the 7 samples identified with *Pseudomonas aeruginosa*, 4 had a reduced sensitivity to quinolones and represented 57.1%. Among the isolates, 38.2% of them expressed resistance to all the quinolones. There was more resistance to nalidixic acid with 59.1% cases. *Escherichia coli* was the species that showed more resistance to all the quinolones tested and was followed by *Klebsiella pneumoniae*.

Cross resistance between different antibiotic families

Among the ESBL phenotype germs, 94.3% manifested a resistance phenotype to at least one quinolone and one aminoglycoside at the same time. 100% of ESBL producing germs manifested a resistance phenotype to at least 3 different families of antibiotics.

DISCUSSION

Of all the isolated enterobacteria, 52.5% were ESBL producers. This ESBL rate is above the 9% observed by Guessennd et al. (2008). This confirms the fact that ESBL producing strains are growing over the years (Guessennd et al., 2008). This proportion is close to that of Obeng-Nkrumah et al. (2013) who obtained 49.3% ESBL producing enterobacteria, and Métuor-Dabiré et al. (2014) who found 56 to 63.3%. This high quantity of ESBL producing bacteria in this study can be justified by the very poor health and environmental hygiene conditions, the lack of a rigorous prescription and access to antibiotics in hospitals and drug stores, the street medicines phenomenon, the high cost of life pushing patients to stop treatment well before the deadline. This high ESBL production of this study species could also be explained by the fact that many of the microorganisms in this study have been isolated in samples of patients who came back for controls after antibiotic treatment. The isolated germs in these cases confirm the failure of the previous treatment and the germs become more resistant. Guessennd et al. (2008) in Côte d'Ivoire suspected the selection constraint used by practitioners and the presence of low concentrations of unmetabolised antibiotics released by the hospital to be a cause of MDR dissemination among which the ESBL producing Gram negative bacteria (Guessennd et al., 2008). A study on the hospitals discharge in Burkina Faso would permit to cast light on this possibility.

The ESBL producing germs according to the bacterial species

Most ESBL were found in *E. coli* and *K. pneumoniae,* both representing 92.4% cases equivalent to 60.4 and 32% cases respectively followed by *Enterobacter sp.* with5.7% cases. The study results are similar to those of are among the commensal enterobacteria of the digestive tract, those that have a great capacity to become pathogenic. *E. coli* remains influential in the urinary tract infections; urine being the source of most of the samples

Table 4. ESBL distribution according to socio-demographic characteristics and nature of product.

Age (months)	ESBL	
	Number (n)	Percentage (%)
1-30	17	32.1
61-120	14	26.4
121-180	9	17.0
< 1	5	9.4
> 180	5	9.4
31-60	3	5.7
Total	53	100
Nature of sample		
Pus	27	50.9
Urine	22	41.5
Blood	4	7.6
Total	53	100
Services		
Surgery	24	57.1
Infant	4	9.5
Older children	4	9.5
Infectious diseases	3	7.2
Neonatology	3	7.2
Others	4	9.5
Total	42	100
Previous antibiotherapy		
Yes	42	79.2
Unknown	8	15.1
No	3	5.7
Total	53	100.0

received, that can explain why E. coli is the most isolated germ and in which more ESBL phenotype was found. *Klebsiella* is an enterobacteria very disseminated in hospitals and is associated to suppuration and to bacteremia (Obeng-Nkrumah et al., 2013).

Pus comes second after urine in the samples and justifies that *Klebsiella pneumoniae* is the second prevalent germ with the highest ESBL production. However, considering every bacterial species, we noticed there was more ESBL production in *Klebsiella pneumoniae* (65.4%), followed by *E. coli* (56.1%). This prevalence was also very prominent in *Enterobacter cloacae* and *Providencia stuartii with* 50% cases.

These figures are as high as those of Obeng-Nkrumah et al. (2013) who found that 75% *Enterobacter* cloacae, 61.5% *K. pneumoniae* and 43.7% *E.coli* showed the ESBL phenotype. Métuor-Dabiré et al. (2014) found superior figures equalling 69.38% for *Klebsiella* sp*,* 65.88% for *E.coli*, 75% for *Enterobacter* sp. and 58.33% for *Pseudomonas.* Also, Lonchel et al. (2012) in Cameroon in 2012 found 66.7% isolates of ESBL producing *E. coli*. This difference of proportions for *Pseudomonas* and the high proportion for ESBL producing *Providencia stuartii* might be explained by the low presence of these species in the bacterial population of our study. An important presence of these species would permit to better appreciate the ESBL production. The difference of proportion of ESBL producing *Enterobacter sp*. compared to the results of Métuor-Dabiré et al. (2014) and Obeng-Nkrumah et al. (2013) can also be justified by the lower quantity of this species in our bacterial population.

The highest ESBL prevalence in this study has been found with *K. pneumoniae.* We observed from many studies that the ESBL prevalence was higher for *K. pneumoniae* than for *E. coli* until the end of the years

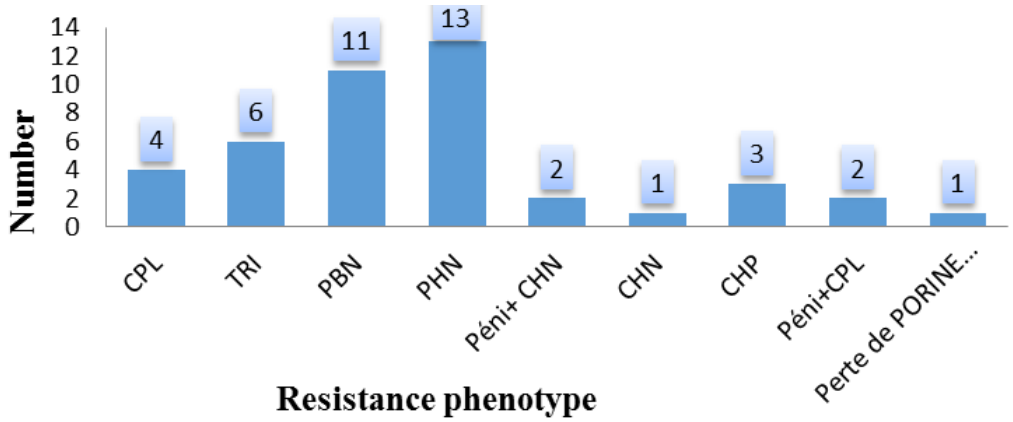

Figure 1. Distribution of the other resistance phenotypes to β-lactams.

1990s (Obeng-Nkrumah et al., 2013; Rodriguez-Villalobos and Struelens, 2006). But, nowadays, there is an immense change of the global epidemiological situation: ESBL producing strains are now mostly *E. coli* that is responsible for community infections and are mainly from urinary samples. This different result compared to our study could be due to the fact that most of our germs were isolated from hospitalised patients.

ESBL producing germs based on status at hospital

The frequency of ESBL production was 24.5% in out-patients against 64.6% in hospitalised ones. These figures are similar to those of Dabiré (2014) in Burkina Faso who reported 17% in out-patients against 83% in hospitalised ones. From these results we can deduce that hospitalisation is a risk factor for ESBL transmission. This observation has been made by Rodriguez-Villalobos and Struelens (2006) in Belgium that extended hospitalisation as a risk factor for ESBL producing germs dissemination.

ESBL producing germs based on the sample nature

100% germs from hemocultures showed ESBL phenotype followed by pus with 58.7% cases and urine with 40%. Ouédraogo et al. (2016) found that 62% urine and 57% pus contained ESBL producing germs. Obeng-Nkrumah et al. (2013) observed that 66.7% urine, 45.6% hemoculture samples and 0% pus contained ESBL producing germs. The primacy of hemoculture containing ESBL producing germs in this study might be explained by the low proportion of germs coming from these samples (3/110). There was an important proportion of ESBL producing germs in pus compared to urine; this result is different from those of Ouedraogo et al. (2011) and Obeng-Nkrumah et al. (2013) who found an ESBL predominance in urine. Most of the pus samples in this study has been taken from hospitalized patients and 86.9% of those patients have taken antibiotics during their hospitalisation just before the sampling for bacteriological test. In addition, the third-generation cephalosporins (C3G) were the most used with Cefriaxone. Different studies show the impact of these antibiotics on the increase of ESBL enterobacteria production (Ouedraogo et al., 2011; Sangare et al., 2015). This phenomenon could explain the highest proportion of ESBL producing germs in pus compared to urine.

Other resistance phenotypes

Among the germs that do not have the ESBL phenotype, we found other resistance phenotypes to bêta-lactams whose impact on the bacterial resistance is not negligible. Indeed, 13 PHN phenotypes, 11 PBN, 6 TRI, 4 CPL, 3 CHP and 1 loss of Orp D2 porine have been recorded. This observation increases the phenomenon of resistance taking into account that the ESBL producing strains come from these penicillinases changes. No carbapenemase producing strains have been found. This is explained by the low availability of carbapenemase in our regions and its high cost; that is why it was found only in previous therapy of only 3 patients among the 106 entering the study which equals 2.8%. Among the germs having the ESBL phenotype, 94.3% manifested a resistance phenotype to at least one quinolone and one aminoglycoside at the same time; 100% of ESBL producing germs manifested a resistance phenotype to at least 3 different antibiotic families. These results confirm the observation by Boyd et al. (2004) who reported that ESBL are carried by large size plasmids which very often contain resistance genes to other antibiotic classes thus

making the host bacterium multi-resistant (Boyd et al., 2004).

Conclusion

From this study, it was observed that *E. coli* was the predominant species mainly found and about half of the isolates (48.2%) were ESBL+. In-patients had more risk to have an ESBL phenotype than out-patients. The high incidence of ESBL-secreting Gram negative bacteria should lead prescribers to improve the quality of their prescription, preferably based on the results of a correctly performed and interpreted antibiogram. The good delivery of antibiotics in officinal medicine and hygiene measures are points on which the focus must be put in order to reduce this global menace of bacterial resistance because despite the constant expansion of this phenomenon, it is also important to note that antibiotics still save millions of lives.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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