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Bloodstream infections initiated by ESBL-producing *Escherichia coli* in neonates and infants at two hospitals in Yaoundé, Cameroon

Carole Suzie Lacmago TITSAMP[1], Hortense Kamga GONSU[2] et Simon Ngamli FEWOU[1*]

[1]*Department of clinical biochemistry, Faculty of medicine and biomedical sciences, Université des Montagnes, Bangangté, Cameroon.*
[2] *Department of microbiology, Faculty of medicine and biomedical sciences, University of Yaoundé I, Yaoundé, Cameroon.*
[*]*Corresponding author; E-mail: simon.fewou@gmail.com*

ABSTRACT

Early diagnosis and probabilistic antibiotic therapy based on known bacterial ecology and antibiotic sensibility can reduce mortality and morbidity in pathologies caused by a bacterial infection. This study aimed at determining the prevalence and risk factors of extended-spectrum β-lactamases (ESBLs)-producing *Escherichia coli* isolated from blood cultures of neonates and infants population. We conducted a cross-sectional study during which pathogenic bloodstream isolates were identified. Antibiotic susceptibility test was performed on *Escherichia coli* isolates and phenotypic confirmation of ESBL production by *Escherichia coli* was performed by a double-disc synergy test. Over the course of this study, 298 blood cultures were performed and 129 (43.3%) positive cultures were obtained. Of the 129 bacterial isolates, 90 (69.7%) were *Escherichia coli* and 39 (30.2%) were other bacteria strains that included *Klebsiella oxytoca*, *Streptococcus pneumonia*, and *Coagulase-negative staphylococci*. Antibiotic susceptibility test indicated that *Escherichia coli* isolates were resistant to cephalosporin, penicillin, sulfonamide, and aminoglycoside antibiotic families. Further analysis indicated that 31 (34.4%) *Escherichia coli* strains were ESBL producers and risk factors for bloodstream infection by ESBL-producing *Escherichia coli* were prior to exposure to antibiotics and immune system depression. These findings clearly extend our understanding of the type of resistant initiated by ESBL-producing *Escherichia coli* in bloodstream infection of neonates, and infants and also provides useful information that can guide the establishment of an efficient therapeutic strategy for the community- and hospital-acquired bloodstream infection.

Keywords: Bloodstream infection, *Escherichia coli*, antibiotic resistance, ESBLs, neonates, risk factors.

INTRODUCTION

The World Health Organization (WHO) has reported approximately 140.87 million newborns in the year 2015 (Roser et al., 2020). Moreover, 2.5 million deaths among newborns within the first four weeks of life were also reported (WHO, 2019). At the current death rate, 30 million newborns will die within the first 28 days of life between 2017 and 2030 (WHO, 2019). The vast majority (30-40%) of

these deaths is due to neonatal infections (THE WHO YOUNG INFANTS STUDY GROUP, 2019).

Neonatal and infant infection is a major cause of morbidity and mortality (Paneth and Thompson, 2018). It affects more than 1% of births and 10 to 15% of infants admitted to an intensive care unit and there are several chronological stages in neonatal infections (Tam and Bendel, 2017). Throughout intrauterine life, the embryo and the fetus are at risk of being affected by various infectious agents carried by the mother. After birth, newborn babies at term and/or premature babies are very vulnerable to infection (Remington and Klein, 1995). Therefore, rigorous hygiene is essential to avoid the postnatal infection. However, the uncontrolled or misuse of antimicrobial molecules may induce resistance, hence increasing the risk of therapeutic failure. Antimicrobials can effectively fight infections and antibiotic therapy is the most widely used control methods for bacterial infections. Used as a preventive or curative, antibiotic therapy has saved a large number of lives. Nowadays antibiotics are less and less effective due to the appearance of resistant strains.

Escherichia coli (*E. coli*) is a bacteria species with versatile characteristics which comprise commensal as well as pathogenic variants that cause diseases in human and animals (Leimbach et al., 2013; Daga et al., 2019). In addition, pathogenic *E. coli* strains express specific proteins that contribute to the emergences of antimicrobial resistance. One of these strains is the multidrug-resistant *E. coli* strain that produces extended-spectrum β-lactamases (ESBL). The occurrence of extended spectrum beta lactamases-producing *E. coli* which initiate treatment failure of cephalosporin therapy has become an important epidemiological event (Wu et al., 2019). ESBL gene confers resistance to various antibiotics including penicillin, aztreonam and cephalosporin. In addition, it could also confers resistance to other antibiotic classes that include aminoglycosides, trimethoprim-sulfamethoxazole and quinolones (Paterson et al., 2000; Oteo et al., 2001; Paterson and

Bonomo, 2005; Rawat and Nair, 2010; Ghafourian et al., 2014). It is demonstrated that the clinical importance of ESBL-producing *E. coli* isolates is relevant due to limited therapeutic options and high risk of treatment failure in bloodstream infected patients (Hyle et al., 2005; Serefhanoglu et al., 2009). It is therefore, important to determine risk factors for the infection with multidrug-resistant ESBL-producing *E.coli* isolates in order to improving the therapeutic strategy.

The objective of this study was to determine the prevalence and risk factors associated with ESBL-producing *E. coli* among infants and neonates at the Yaoundé University Hospital Center and Yaoundé Gynecology, Obstetrics and Pediatrics Hospital, two hospitals in city of Yaoundé. The results obtained at the end of this study may contribute to develop an efficient therapeutic strategy for the treatment of community- and hospital-acquired bloodstream ESBL-producing *E. coli* infection by neonatal patients.

MATERIALS AND METHODS
Sample collection and bacteria identification

The cross-sectional study was carried out in the pediatric department of the Yaoundé Gynecology, Obstetrics and Pediatrics Hospital (YGOPH) and Yaoundé University hospital center (YUHC) between August and October 2017. Patients between 0 and 42 weeks of age visiting the neonate service for consultation due to sepsis suspicion as well as other infectious diseases of bacterial origin or those hospitalized were included in this study. Before any blood test, parents first filled out a survey sheet containing questionnaires to measure specific parameters. Thereafter, venous blood sample between 1 to 5 mL were collected and immediately inoculated in a pediatric blood culture bottle containing trypticase soy broth supplemented with growth agents, reducing agents and anticoagulant (Oxoid Limited, Thermo Fisher, Hampshire, England), and incubated at 37 °C. Gram staining test was also performed from positive blood samples and a subculture on Mac

Conkey agar medium was done for the selection of *Enterobacteria*. The identification of bacteria strains was done using the galleries API 20E system (BioMérieux SA, Marcy l'étoile, France) as described by the manufacturer instructions.

Antibiotic susceptibility test

The antibiogram was performed by the diffusion method using antibiotic discs in agar medium on Mueller Hinton agar according to the Kirby Bauer technique (CA-SFM, 2014). Fifteen (15) antibiotics (BioMérieux SA, Marcy l'etoile, France) that include amoxicillin (30 µg), amoxicillin + clavulanic acid (30 µg; Augmentin), cefotaxime (30 µg), ceftazidine (30 µg), cefepime (30 µg), cefoxitin (30 µg), amikacin (30 µg), nalidixic acid (30 µg), ertapenem (30 µg), ciprofoxacin (30 µg), ofloxacin (30 µg), fosfomycin (30 µg), kanamycin (30 µg), cotrimoxin (30 µg) and gentamycin (30 µg) were used. The inoculums were prepared from an 18-24 hour pure colony culture in sterile physiological water (0.85% NaCl). The suspension was standardized to 0.5 Mac Farland. The results were interpreted according to the criteria of the "Comité d'Antibiogramme de la Société Française de Microbiologie" (CA-SFM, 2014).

Screening of ESBL producing strains of *E. coli*

The screening test for the production of ESBL was performed using both ceftazidime (CAZ) (30 µg) and Ceftriaxone (CTR) (30 µg) disks. If the zone of inhibition was less than or equal to 22 mm for CAZ and/or less than or equal to 25 mm for CTR, the *E. coli* isolate was considered a potential ESBL-producer as recommended by the Clinical and Laboratory Standard Institute (CLSI, 2014). To confirm the production of ESBL, susceptible *E. coli* bacteria screened for ESBL production were subjected to combined disk diffusion test using CAZ (30 µg), alone and in combination with clavulanic acid (CA) (10 µg). After overnight incubation at 37 °C, if the zone of inhibition was higher or equal to 5 mm for both antibiotic tested and in combination to clavulanate, the *E.*

coli strain considered as positive for ESBL production.

Data management and statistical analysis

Data extracted were entered in Microsoft Excel 2010 spreadsheet and the variables presented as percentage. The statistical analysis of all data was done using the IBM Statistical Package for the Social Sciences version 11.0 (SPSS Inc, Chicago, USA). The antibiotic susceptibility and frequency of infections caused by ESBL-producing *E. coli* was reported as the number of infections per 100 positive patients. Univariate and multivariate analysis were performed using logistic regression. Multivariate analysis of characteristic features for bloodstream infection of ESBL-producing *E coli* included the following variables; three months hospitalization, antibiotic exposure, immune depression and blood transfusion. To assess the risk factors associated with ESBL-producing *E. coli*, the categorical variables were compared using the Fischer test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated on the Open Epi Info website (www.openepi.com). The multivariate analysis was performed using a conditional logistic regression model (SAS University Edition, SAS Institute Inc., Cary, USA) by including the variables suggested by the univariate analysis (p <0.1). All tests were bilateral and $p < 0.05$ was considered to be statistically significant.

Administrative and ethical consideration

This study was approved by the Medical Ethical Committee of the "Université des Montagnes" (2017/155/UdM/PR/CAB/CIE). In addition, Permissions to collect data and samples from eligible patients were granted by the Yaoundé University Hospital Center (783/AR/CHUY/DG/DGA/DMT) and Yaoundé Gynecology, Obstetrics and Pediatrics Hospital (580/CIERSH/DM/2017). Prior to entering the study, interested parents received verbal and written information on the objectives and procedure of the study. All eligible parents were provided informed

consent according to the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. In addition, each parent signed the informed consent form for the infants or neonates patients. The confidentiality of the research results was respected by the use of a unique research identity number for each participant.

RESULTS
Demographic characteristics and bacterial isolates

During the study period, 298 patients aged 0 to 42 weeks old were selected among the 660 children present. Patients participating to this study were either attending the hospital for consultation or simply hospitalized because of bacterial infection. More than half (81.9%; n=244) of the patients were between 0 to 4 weeks (neonates) of aged while 61% (n=182) were between 0 and 1 week old and 41.9% (n=125) were one day old or less. Among these patients, 51% (n=152) were male and 49% (n=146) female and most of them were patients who came to the hospital for consultation. Furthermore, 65% of the patients were from the pediatric department of YGOPH and 35% from the Yaoundé University hospital center (YUHC). Blood culture test showed that 129 (43.3%) samples were positive and indicated the presence of bacterial infection.

Isolation and Identification of bacterial strains

Screening of the 129 positive blood samples using the antibiotic susceptibility method allowed the isolation of 129 strains among which 69.77% (n=90) were *E. coli* and 39 (30.23%) bacterial isolates were from other strains which includes *Klebsiella oxytoca*, *Streptococcus pneumoniae* and *Coagulase-negative staphylococci* (Table 1). Of the 90 *E. coli* isolates, 39 (43.3%) were form the blood samples of patients aged one day or less, while 65 (72.22 %) were found in the blood samples of newborns patients aged 0 to 4 weeks and 25 (27.77%) were from the blood samples of patients aged between 4 to 42 weeks. Further analysis showed that each patient was colonized by one type of micro-organism.

Characterization of the *E. coli* strains

All *E. coli* isolates (n=90) were characterized by the disc diffusion method using 7 antibiotic of the ß-lactam family (two penicillin's, four cephalosporin's and one carbapenems). The results are shown in Table 2 and highlight a higher resistance rate (85.5%) of *E. coli* to two antibiotics (amoxicillin and augmentin) of the penicillin family. In addition, *E. coli* exhibited an average resistance to three antibiotics of the cephalosporin family, namely ceftazidime (65.55%), cefepime (58.88%) and cefoxitin (55.55%). Moreover, 67.8% (n=61) of the isolated *E. coli* strains were resistant to third generation cephalosporin (ceftazidime and cefotaxime), while 30% (n=27) were resistant to second generation cephalosporin (cefoxitin) and 58.88% (53/90) were resistant to fourth generation cephalosporin (cefepime).In contrast, some *E. coli* isolates showed a low resistance rate (30%, 24.44%) when the cephalosporin cefotaxim and the carbapenems, meropenem were used as antibiotic, respectively (Table 2).

The susceptibility of *E. coli* isolates to other antibiotic families was also tested (Table 3). In the first instance, the antibiotic susceptibility of *E. coli* isolates was tested using 3 antibiotic discs belonging to the quinolones family. Results of these tests indicated that the *E. coli* isolates were weakly resistant to ciprofloxacin (36.66%), nalidixic acid (34.44%) and ofloxacin (27.77%), three antibiotics of the quinolones family. In contrast, the antibiotic susceptibility test of the *E. coli* isolates provided a significantly higher resistance rate when the antibiotic of the sulfonamides family, co-trimoxazole (92.2, n=83) was used (Table 3). Likewise, a higher resistance rate in similar test was also observed when an antibiotic of the aminoglycoside family (Amikacin, 54.44%; Gentamicin, 57.77%; Kanamycin, 98.88%) was used (Table 3).

Extended spectrum β-lactamases (ESBL)-producing phenotypes among *E. coli* isolates

To further characterize the 90 strains of *E. coli* isolates obtained in this study, the strains that express the ESBL phenotype was identified. Therefore, the double disc approximation test was performed. This

method allowed to detecting the production of broad-spectrum β-lactamases by 31 *E. coli* isolates out of a total of 90, representing a prevalence rate of 34.44%. Moreover, 35.5% (11/31) of *E. coli* isolates expressing the ESBL phenotype were isolated from the blood sample of patients aged one day or less, while 9.7% (03/31) of *E. coli* isolates expressing the ESBL phenotype were isolated from the blood samples of patients aged 1 to 7 days and 32.3% (10/31) of *E. coli* isolates expressing the ESBL phenotype were isolated from the blood samples of patients aged between 1 to 4 weeks. In summary, 83.3% (25/31) of the *E. coli* isolates expressing the ESBL phenotype were from neonatal patients, which is considered as the period beginning at birth and ending at 28th days (WHO, 2006; Pathirana et al., 2016). Four (4) (12.9%) *E. coli* isolates with ESBL phenotype were found in the blood samples of patients aged 4 to 12 weeks, while the blood samples of patients aged between 3 to 10 months old showed 9.7% (3/31) frequency of *E. coli* isolates with ESBL phenotype.

Risk factors associated with ESBL-producing *E. coli* isolates

Multivariate logistic regression analysis revealed that hospitalization was not a risk factor for infection by ESBL-producing *E. coli*. Multivariate regression analysis indicated that prior exposure to antibiotics was associated with risks of infection by ESBL-producing *E. coli* (OR = 1.97; 95% CI= 1.02; 3.83; *p*= 0.04). A more detailed analysis indicated that among the tested antibiotics, only antibiotics of the β-lactams families was likely to associated the higher risk of infection with ESBL-phenotype by *E. coli*(OR = 3.54; 95% CI= 1.28-9.80; *p*= 0.014) (Table 4). Further analysis showed that condition such as immune depression was a significant risk factor associated with the infection by ESBL-producing *E. coli* (OR = 19.93; 95% CI =8.82; 45.32; *p*= 0.0001), while blood transfusion (OR =2.48; 95% CI =0.95-6.45; *p*=0.06) was not (Table 4).

Table 1: Microbiological profile of the bacterial isolates.

Bacteria isolates	Size (n)	Percentage (%)
Escherichia coli	90	69.77
Other bacteria strains	39	30.23
Klebsiella oxytoca	-	-
Streptococcus pneumoniae	-	-
Coagulase-negative staphylococci	-	-

Table 2: Susceptibility of *E. coli* isolates to antibiotics of ß-lactams family.

Antibiotic family	Antibiotics	Resistance	
		Sample size (n)	Percentage (%)
Cephalosporin's	Cefepime	53	58.88
	Ceftazidime	59	65.55
	Cefotaxime	50	55.55
	Cefoxitin	27	30
Penicillin's	Amoxicillin	77	85.55
	Amoxicillin + clavulanic acid	77	85.55
Carbapenem	Meropenem	22	24.44

Table 3: Susceptibility of *E. coli* isolates to other antibiotics families.

Antibiotic family	Antibiotics	Resistance	
		Size (n)	Percentage (%)
Sulfonamides	Cotrimoxazole	83	92.2
Quinolones	Ciprofloxacin	33	36.66
	Nalidixic acid	31	34.44
	Ofloxacin	25	27.77
Aminoglycosides	Amikacin	49	54,44
	Gentamicin	52	57.77
	Kanamycin	89	98.88

Table 4: Multivariate analysis on risk factors for the bloodstream infection by ESBL-producing *E. coli*.

Variables	Odds ratio(OR)	95% confidence interval (CI)	*p*-value
Three months Hospitalization			
No	1.0		
Yes	2.94	0.97; 8.91	0.06
Prior exposure to antibiotics			
No	1.0		
Yes	1.97	1.02; 3.83	0,04
β-lactams			
No	1.0	.	.
Yes	3.54	1.28; 9.80	0.01
Amino glycosides			
No	1.0		
Yes	1.0	1.0; 1.33	0.41
Co morbidities			
Immune depression	19.93	8.82; 45.32	0.0001
Blood transfusion	2.48	0.95; 6.45	0.06

DISCUSSION

The management of septicemia and other pathologies caused by bacterial infections in children is based on urgent and adequate presumptive antibiotic therapy. The choice of molecules used requires knowledge of local bacterial ecology and the sensitivity of germs to antibiotics. It is for the specific purpose of improving the management of infectious diseases of bacterial origin that the rate and risk factors of extended spectrum β-lactamases (ESBLs)-producing *E. coli* isolated from bloodstream cultures of neonates and an infant at two hospitals in Yaoundé (Cameroon) was estimated. The results of this study revealed that the average combined prevalence of infection with ESBL-producing *E. coli* among these patients at both hospitals in high. Further analysis showed that risk factors for infection with ESBL-producing *E. coli* included prior exposure to antibiotics of β-lactams family and immune depression. The neonatal infection reported here may be caused by mother to child infection during childbirth since it happens early in child life as already reported by other investigation (Kari et al., 2014). The current study may contribute to the improvement therapeutic strategy against EBSL-related diseases in Cameroon and Africa in general.

The overall bacterial profile in this study revealed a predominance of *E. coli* (69.8%; n=90) as the major bacterial specie and the remaining composed of 30.2% (n=39) *Klebsiella oxytoca*, *Streptococcus pneumonia* and *Coagulase negative staphylococci*. This finding is similar to that described by Chiabi et al. in Bertoua (2005) and Yaoundé (2011), Cameroon. In addition, similar data have also been reported in countries like Morocco (Chemsi and Benomar, 2015), Tunisia (Ben et al., 2013) and India (Manta et al., 2015). Further investigations using antibody susceptibility test indicated a higher prevalence of *E. coli* resistance toward amoxicillin/ amoxicillin added to clavulanic acid, two antibiotics of the penicellin's family. In addition, higher *E coli* resistant rate toward antibiotics third generation cephalosporin was also observed. It is important to mention that increased resistance to third generation cephalosporin (3GC) is a serious concern for community-onset *E. coli* infection in Africa because this antibiotic is easily accessible to population. In this condition, delay of effective treatment is observed because of misuse of antibiotic, therefore leading to increased morbidity and mortality in the concerned population. Other studies from around the world have already reported similar resistance rates of *E. coli* to third generation (Gbaguidi-Haore et al., 2013; Lin et al., 2019; Lester et al, 2020). Likewise, *E. coli* isolates in this study displayed a resistance to cotrimoxazole and kanamycin, two antibiotics of the sulfonamide and aminoglycoside family, respectively. Together with the resistance observed toward the 3GC antibiotic, this suggests a limited therapeutic strategy to cure bacterial infections that affects the investigated patients at both hospitals. In addition, studies from around the world have reported similar resistance rate (Le Doare et al., 2015; de Oliveira et al., 2019).

Infections initiated by the enzyme ESBL-producing *Enterobacteriaceae* are resistant to a broad range of β-lactams, including 3GC. Among all the pathogens, *E. coli* has emerged as one of the world's greatest health threats in past two decades (Padmini et al., 2017). The high rate of ESBL-producing *E. coli* strains found in this study should be interpreted with caution. But potential reasons may be related to the fact that when infections are suspected for the first time in primary care unit, antibiotics may be prescribed without drawing any biological sample since microbiology laboratories are not well equipped and lack most of the reagents needed to perform a proper diagnostic. Indeed, antibiotic prescriptions prior to blood culture are also reported in pediatric care units of other African countries (Blomberg et al., 2007; Ndir et al., 2016). In addition, other interpretation for the high rate of ESBL-producing *E. coli* observed in this study may also implicate the overuse and misuse of antibiotic drugs prior to hospitalization and antibiotic prescribing errors as also reported elsewhere (Davies and Davies, 2010; Hosoglu et al., 2013; Abera et al., 2014; Shiva et al., 2018). It is also important to mentioning that consumption of food-

containing antimicrobial substances like pesticides may also be one of reasons for the appearance of antimicrobial resistance in infants and neonates in this study since other publications have reported that pesticides consumption through may initiate antibiotic resistance in bacteria (Bell et al., 2014; Gondam et al., 2016).

In this study, hospitalization and blood transfusion were not significantly associated with increased risk for ESBL-producing *E. coli* infection. In contrast, antibiotic misuse was strongly associated with risk for infection by antibiotic resistant *E. coli*. Although exposure to several antibiotic classes prior to hospitalization or physician consultation were significant on univariate statistical analysis, only exposure to β-lactams antibiotic family remained statistically significant in the multivariable regression models. In addition, it is demonstrated that children who had exposure to β-lactams antibiotic family had three to four times greater risk for ESBL-associated infection (Zaoutis et al., 2005; Kuo et al., 2007; Topaloglu et al., 2010; Kizilca et al., 2012; Flokas et al., 2016; Li et al., 2018; Hu et al., 2019). The high rates of β-lactams resistance observed here complicate the management of the disease initiated by infection with ESBL-producing *E. coli*, therefore lead to the use of other antibiotic family and thus considerably increasing the cost of treatment. Similar findings are reported by other investigators (Tenover et al., 1999; Kim et al., 2002; Zaoutis et al., 2005; Ndir et al., 2016). Another risk factors for bloodstream infection observed in this study was immune depression which was significant associated with high risk infection with ESBL-producing *E. coli* in children.

Conclusion

It was noticed that out of 129 bacteria isolated from newborm bloodstream, 90 were *E. coli*. In addition, a large number of the isolated *E. coli* strains showed a significantly high resistance to cepholosporins, penicillins, sulfonamides and aminoglycosides. Furthermore, the high rate of ESBL-producing *E. coli* obtained in this study is an emergent

cause of neonatal death locally recorded. It was also found that previous exposure antibodies and more specifically to antibiotics of the β-lactams family and a preexisting immune depression condition were risk factors associated to the infection to ESBL-producing *E. coli*. From these findings, there is reason to be concerned about the installation of the ESBL plasmids encoding this species in neonatal population in both healthcare institutions. This work should make it possible to adapt the probabilistic antibiotic therapy of bacteremia, and to set up a strategy to control the development and spread of multi-resistant bacteria. Reinforced hygiene measures in hospitals and communities could reduce bacteremia with multi-resistant germs.

COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

SNF conceived and designed the study. Patient recruitment and data collection were done by CSLT. SNF, HKG and CSLT conducted the data analysis. SNF, HKG and CSLT interpreted study results. SNF wrote the manuscript. All authors approved the final copy.

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