Original Research Article

Screening for Beta-lactamases and other resistant bacteria among patients admitted to the Emergency unit at the Yaoundé University Teaching Hospital

ABSTRACT

Aims: This study aimed to screen for Beta Lactamases (BLs) and other resistant bacteria among patients admitted to the emergency unit of the Yaoundé University Teaching Hospital (YUTH).
Study design: This study was across-sectional hospital-based study.
Place and Duration of Study: This study was conducted at the Bacteriology unit of the YUTH, Yaoundé- Cameroon between February to June 2020.
Methodology: Seventy-Five urine samples were collected from newly admitted patients at the emergency unit of the YUTH and bacteria species were identified basis on their culture characteristics, Gram morphology, and biochemical tests. The isolates were screened from the production of extended-spectrum beta-lactamases (ESBLs) and AmpC BLs using the Double disk synergy method and Disk approximation methods respectively. These isolates were later subjected to antimicrobial susceptibility testing using the disk diffusion method.
Results: Out of the 75 urine analyzed, 14 (18.7%) were found positive for Urinary Tract Infection. Fourteen bacteria species were isolated identified and enumerated as *E.coli* (5), Klebsiella species (4), *Citrobacter species* (2), *Proteus species* (2), and *Enterobacter species* (1). Ahigh level of resistance was observed with Amoxicillinclavulanic acid, Cefuroxime, and Ceftazidime while a high level of sensitivity was observed among carbapenem antibiotics. Eight of 14 isolated bacteria were BLs producers, of which 5 were sorely ESBL producers, 2 co-producers (ESBL + AmpC), and 1 AmpC producer. The overall positive rate of BLs in the study population was 10.7%. Again, patient origin and previous antibiotic use were significantly associated with BLs prevalence p-value of .01 and .04 respectively.
Conclusion: The high prevalence of the ß- lactamases in the Emergency unit emphasizes the need for continuous surveillance in the Emergency unit to detect resistant strains, strict guidelines for antibiotic therapy, and the implementation of infection control measures to reduce the increasing burden of antibiotic resistance.

Keywords: Beta-lactamase, Prevalence, Urinary tract infection, Extended Spectrum Beta-Lactamases, AmpC Beta-Lactamases.

1. INTRODUCTION

Beta-lactam antibiotics, such as monobactams, cephalosporins and carbapenems, are the important antibiotic for the treatment of bacterial infections [1]. The main mechanism of resistance to these antibiotics is linked to the production of beta-lactamase (BL) hydrolysing enzymes such as extended-spectrum beta-lactamases (ESBL), AmpC beta-lactamases and carbapenemases [2]. Originally, BLs were mainly demonstrated in bacteria isolated from patients hospitalized in intensive care units. Epidemics, caused by these bacteria, starting in

the intensive care units and spreading to other parts of the hospital have been well documented [3]. Later on, BLs isolated from humans with urinary tract infections (UTIs) in the community became more frequently described. Most of these isolates are not only resistant to ceftriaxone, but also to other commonly used first-line agents for UTIs, such as trimethoprim-sulfamethoxazole, ciprofloxacin, gentamicin, and nitrofurantoin [3]. The most frequent isolated group of bacteria producing BLs are Enterobacteriaceae. However, factors such as age, gender, previous UTI, exposure to antibiotics, previous hospitalization, urinary or arterial catheterization, surgical operation, and underlying diseases such as diabetes and cardiovascular, predispose individuals to these BLs-producing bacteria [4].
Therefore, monitoring the prevalence of BLs producers at a local, regional, or global level is required to develop optimal ways to adapt clinical practices and to determine the most effective agents and strategies for the treatment of infections caused by these bacteria. Several studies on antimicrobial resistance have been carried out in and out of Cameroon, but few on beta-lactamase-resistant strains have been published. The purpose of this study is to screen for beta-lactamases and other resistant bacteria among patients admitted at the emergence at the YUTH. This research will contribute to the knowledge of the local prevalence of beta-lactamase strains and other resistant bacteria from patients coming from the community and other hospital settings. It will also contribute to ensuring appropriate treatment of patients and prevent further development of drug resistance.

2. MATERIAL AND METHODS

2.1 Study area and period

This study was carried out at the Yaoundé University Teaching Hospital (YUTH) from February to June 2020. YUTH is located in the Central Region of Cameroon. YUTH is one of the 4 reference hospitals in Yaoundé, handling the diagnosis, treatment, and management of many diseases and also the handling of referred patients coming from different hospitals. The emergency unit of the said hospital is made up of 25beds managed by a head of the service who is an internist/cardiologist, 5 general practitioners' doctors, and 25 nurses including the Hygiene and Security staff.

Comment [F1]: No need for such elaboration about the hospital structure, what should be mentioned is the privience from which patients came from.

2.2 Study design and population

We carried out a hospital base cross-sectional study enrolling newly admitted patients at the emergency unit of YUTH The study involved patients admitted at the emergency unit. The Emergency unit provided us with two groups of patients; Community and hospitalised patients. Hospitalised patients were patients coming from different hospitals, health centers, and clinics. Community patients were patients coming directly from their houses. Patients were recruited using a convenient sampling method.

2.3 Sample processing and Identification of isolates

Mid-stream urine samples were collected from newly admitted patients in the Emergency unit of Yaoundé University Teaching Hospital. All urine samples were aseptically inoculated with C.L.E.D agar and EMB agars using the calibrated loop technique. After overnight incubation at 37˚C for 18 - 24 hours, colony counts yielding bacterial growth of 10^5/ml of urine were regarded as significant for bacteriuria. Identification of bacterial isolates was carried out using biochemical tests.

2.4 Antibacterial sensitivity

Antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method was done following recommendations from the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2019) [5] and inhibition diameters were reported Discs tested included; Cefuroxime (30µg), Amoxicillin-clavulanic acid (30µg), Meropenem (10µg), Ceftriaxone (10µg), Cefoxitin (30µg), Ceftazidime (30µg), Aztreonam(30µg), Imipenem (10µg), Ciprofloxacin (5µg), Gentamicin (10µg).

2.5 Detection of phenotypic resistance

2.5.1 ESBL Detection

Tested isolates have a zone of inhibition less than or equal to 22mm for Ceftazidime and/or less than or equal to 25 mm for Ceftriaxone, the isolate was considered a potential ESBL-producer. The Double Disk Synergy Test was used to confirm the production of ESBLs. It was done by placing discs containing cephalosporins (cefotaxime, ceftazidime) 15 mm to the disc with clavulanic acid (amoxicillin-clavulanic acid). A positive result was indicated when the inhibition zones around any of the cephalosporin discs increased in the direction of the disk containing clavulanic acid. This phenomenon is often referred to as the “KEYHOLE” effect or “CLAVULANIC” effect and was a confirmation of ESBL production.

2.5.2 Detection of AmpC Beta lactamases

Cefoxitin was used to screen for AmpC Beta lactamases production. Resistance to Cefoxitin was indicative of an AmpC strain. A confirmation test was done using the Disc Approximation test wherethe ceftazidime disk was placed at the center of the plate surrounded by substrates (imipenem, cefoxitin, and amoxicillin-clavulanic acid disc). A positive test was any obvious blunting or flattening of the zone of inhibition between the ceftazidime disk and the inducing substrates

2.6 Statistical Analysis

Data analysis was carried out using Microsoft Excel 2016 and SPSS version 25, wherethe chi-square was used to determine the relationship between the variables and their significance.

3. RESULTS AND DISCUSSION

3.1 Sociodemographic data

The participants in this study were categorized with respect to their socio-demographic factors in table 1. at the end of this study, the age of the participants ranged from 14years to 82years and the mean age was 47.5 (standard deviation= 18.8) years. More than half of the participants came from the community and were of the male gender.

3.2Prevalence of Urinary tract infection

After analysis, it was found that 18.7% (n=14) of the participants had UTI and 90.3% (n=61) were found free UTI. This finding is higher than the 11.4% reported by Mchomvu *et al.,* [6] in sub-Saharan African emergency departments. This could be due to differences in demography and rate of antibiotic usage. UTI was higher among males 57.1% (8/14) than females 42.9% (6/14) participants. Lower in community patients 21.4% (3/14) than in

patients coming from hospitals 78.6% (11/14). Again, there was a significant association between urinary catheters and the prevalence of UTI (*P*=0.004) (Table 1).

3.3 Proportion of Beta-lactamase producer

Bacteria were detected in 14 (18.7%) out of 75 analysed, the bacteria isolated included *Escherichia coli* [5/14], *Klebsiella spp* [4/14], *Proteus spp* [2/14], *Citrobacter spp* [2/14] and *Enterococcus spp* [1/14]. *Escherichia coli* and *Klebsiella spp* were the most common isolated species, this finding correlates with other studies in Cameroon [8, 9]. Beta-lactamase production was observed in 8 (72.7%) out of 11 strains of isolated Enterobacteriaceae. Consequently, we had a beta-lactamase positivity rate of 10.7% (8/75) among the total study population, which is similar to that reported by Yusurf *et al.,* [7] in Nigeria of 10.3%. Also, overall 7 isolates were ESBLs producers, making a positive rate for ESBLs production of 9.33%. This is similar to that reported by Liu et al., [8] of 9.34%. Of the 8 positive beta-lactamases, 5 (62.5%) were ESBL producers, followed by 2 (25%) co-producers (ESBL + AmpC) and 1 (12.5%) AmpC producers (Table 2).

3.4 Characteristics associated with Beta-lactamase urinary tract infection

The patient's characteristics are represented in table 3 according to the presence of BLs-producing Enterobacteriaceae and BLs non-producers. There was a significant association between patient origin and BLs production (*P*=0.01) where all the BLs producers came from hospitals. This could be explained by the fact that these patients are greatly exposed to broad-spectrum antibiotics during their hospitalization. Also, there was a significant association between patients' previous exposure to antibiotics (*P*=0.04).

3.5 Antibiotic susceptibility testing

Overall, 11 (78.6%) were found resistant to Cefuroxime. 71.4% (n=10) of the uropathogens were resistant to Amoxicillin-clavulanic acid and Ceftriaxone. However, Imipenem and meropenem were highly effective against the tested uropathogens, with a sensitivity of 78.6% and 64.3% respectively as shown in table 4. This study is supported by studies by Lonchel *et al.,* [9] and by Bissong *et al.,* [10] in Cameroon. Concerning isolates, *E. coli* presented a high resistivity against Amoxicillin clavulanic acid, cefuroxime, ceftriaxone, and ceftazidime). *Klebsiella spp* was highly resistant to cefuroxime, ceftriaxone, and ceftazidime. The antibiotic susceptibility profile of other uropathogen is presented in table 4.

Figure 1 shows the antibiotic susceptibility patterns of the isolated BLs producers and non-producers. BLs producers were found to have higher resistance to beta-lactam antibiotics (up to 87.5%) and other antibiotics, as compared to non-producers. BLs producers were sole to show resistance against meropenem and both presented the same level of resistance to amoxicillin-clavulanic acid. However, imipenem was the only antibiotic to retain its activity in all the isolates, these findings corroborate with that of Lonchel *et al*.,[9] in Cameroon, where the imipenem was only found highly effective and the isolated BLs producers were highly resistant as compared to non-producers.

Table 1: Characteristics of the study population

Characteristics	Study population	UTI positive (N= 14)	UTI negative (N= 61)	*P*-value
Gender				
Male	49	9 (18.4)	40 (81.6)	.93
Female	29	5 (19.2)	21 (80.8)	

Age range (in years)				
<20	2	0	2 (100)	
20- 39	28	4 (14.3)	24 (85.7)	.31
40- 59	18	2 (11.1)	16 (88.9)	
>60	27	8 (29.6)	19 (70.4)	
Entry With Fever				
Yes	27	2 (7.4)	25 (92.6)	.06
No	48	12 (25)	36 (75)	
Patient origin				
Community	39	4 (10.3)	35 (89.7)	.05
Hospital	36	10 (27.8)	26 (72.2)	
Urinary Catherization				
Yes	20	8 (40)	12 (60)	**.004**
No	55	6 (10.9)	49 (89.1)	
Respiratory assistance				
Yes	10	2 (20)	8 (80)	.91
No	65	12 (18.5)	53 (81.5)	
Past hospitalization				
Yes	53	12 (22.6)	41 (77.4)	.17
No	22	2 (9.1)	20 (90.9)	
Operation (within the last 30 days)				
Yes	14	3 (21.3)	11 (78.6)	.77
No	61	11 (18)	50 (82)	
Past UTI				
Yes	8	2 (25)	6 (75)	.69
No	45	7 (15.6)	38 (84.4)	
Unknown	22	5 (22.7)	17 (77.3)	
Previous Antibiotic use (within the last 3 month)				
Yes	42	9 (21.4)	33 (78.6)	.49
No	33	5 (15.2)	28 (84.8)	
Underlying diseases				
Yes	42	8 (19)	34 (81)	.92
No	33	6 (18.2)	27 (81.8)	

Table 2: Distribution of various BLs in isolated uropathogens.

Organisms	BLs Negative	ESBL	AmpC BLs	ESBL+ AmpC BLs	Total BLs Positive (%)
E.coli (n=5)	3	1	0	1	2(25%)
Klebsiella spp(n=4)	1	2	0	1	3(37.5%)
Citrobacter spp (n=2)	0	1	1	0	2(25%)
Proteus spp (n=2)	2	0	0	0	0
Enterobacter spp (n=1)	0	1	0	0	1(12.5%)
Total	**6**	**5(62.5%)**	**1(12.5%)**	**2(25%)**	**8(100%)**

BLs: Beta lactamase; ESBL: Extended Sprectrum Beta-lactamase

Table 3: Univariate analysis of associated risk factors for BLs

Characteristics	Study population	BLs positive (N= 8)	BLs negative (N= 6)	*P*-value
Gender				
Male	9	5 (55.6)	4 (44.4)	.87
Female	5	3 (60)	2 (40)	
Age range (in years)				
<20	0	0	0	
20- 39	4	2 (50)	2 (50)	.89
40- 59	2	1 (50)	1 (50)	
>60	8	5 (62.5)	3 (37.5)	
Patient origin				
Community	4	0	4 (100)	**.01**
Hospital	10	8 (80)	2 (20)	
Entry with fever				
Yes	2	0	2 (100)	.08
No	12	8 (66.7)	4 (33.3)	
Urinary Catherization				
Yes	8	5 (62.5)	3 (37.5)	.64
No	6	3 (50)	3 (50)	
Respiratory assistance				
Yes	2	0	2 (100)	.08
No	12	8 (66.7)	4 (33.3)	
Past hospitalization				
Yes	12	7 (58.3)	5 (41.7)	.82
No	2	1 (50)	1 (50)	
Operation (with the last 30 days)				
Yes	3	2 (66.7)	1 (33.3)	.71
No	11	6 (54.5)	5 (45.5)	
Past UTI				
Yes	2	2 (100)	0	
No	7	5 (71.5)	2 (28.6)	.09
Unknown	5	1 (20)	4 (80)	
Previous Antibiotic use (with the last 3 month)				
Yes	9	7 (77.8)	2 (22.2)	**.04**
No	5	1 (20.0)	4 (80.0)	
Underlying diseases				
Yes	8	5 (62.5)	3 (37.5)	.64
No	6	3 (50)	3 (50)	

Table 4: Antibiotic susceptibility testing of Isolated uropathogens

Antibiotics	Result	Bacteria (n)					
		E.coli	*Klebsielle spp*	*Citrobacter spp*	*Proteus spp*	*Enterobacter spp*	Total

Amoxicillin clavulanic acid	**R**	80.0 (4)	50.0 (2)	100.0 (2)	50.0 (1)	100.0 (1)	71.4 (10)
	S	20.0 (1)	50.0 (2)	0	50.0 (1)	0	28.6 (4)
Cefuroxime	**R**	80.0 (4)	75.0 (3)	100.0 (2)	50.0 (1)	100.0 (1)	78.6 (11)
	S	20.0 (1)	25.0 (1)	0	50.0 (1)	0	21.4 (3)
Ceftriaxone	**R**	100.0(5)	75.0 (3)	50.0 (1)	0	100.0 (1)	71.4 (10)
	S	0	25.0 (1)	50.0 (1)	100.0 (2)	0	28.6 (4)
Ceftazidime	**R**	60.0(3)	75.0 (3)	50.0 (1)	0	100.0 (1)	57.1 (8)
	S	40.0(2)	25.0 (1)	50.0 (1)	100.0 (2)	0	42.9 (6)
Cefoxitin	**R**	40.0 (2)	50.0 (2)	50.0 (1)	0	0	35.7 (5)
	I	40.0 (2)	25.0 (1)	50.0 (1)	50.0 (1)	100.0 (1)	42.9 (6)
	S	20.0 (1)	25.0 (1)	0	50.0 (1)	0	3 (21.3)
Meropenem	**R**	0	25.0 (1)	50.0 (1)	0	0	14.3 (2)
	I	20.0 (1)	50.0 (2)	0	0	0	21.4 (3)
	S	80.0 (4)	25.0 (1)	50.0 (1)	100.0 (2)	100.0 (1)	64.3 (9)
Aztreonam	**R**	40.0 (2)	50.0 (2)	100.0 (2)	50.0 (1)	100.0 (1)	57.1 (8)
	I	20.0 (1)	25.0 (1)	0	0	0	14.3 (2)
	S	40.0 (2)	50.0 (2)	0	50.0 (1)	0	28.6 (4)
Gentamicin	**R**	40.0 (2)	75.0 (3)	50.0 (1)	50.0 (1)	100.0 (1)	57.1 (8)
	I	20.0 (1)	25.0 (1)	50.0 (1)	0	0	21.4 (3)
	S	40.0 (2)	0	0	50.0 (1)	0	21.4 (3)
Ciprofloxacin	**R**	60.0(3)	50.0 (2)	50.0 (1)	0	100.0 (1)	50.0 (7)
	I	20.0 (1)	25.0 (1)	0	50.0 (1)	0	21.4 (3)
	S	20.0 (1)	25.0 (1)	50.0 (1)	50.0 (1)	0	28.6 (3)
Imipenem	**I**	20.0 (1)	0	0	50.0 (1)	100.0 (1)	21.4 (3)
	S	80.0 (4)	100.0 (4)	100.0 (2)	50.0 (1)	0	78.6 (11)

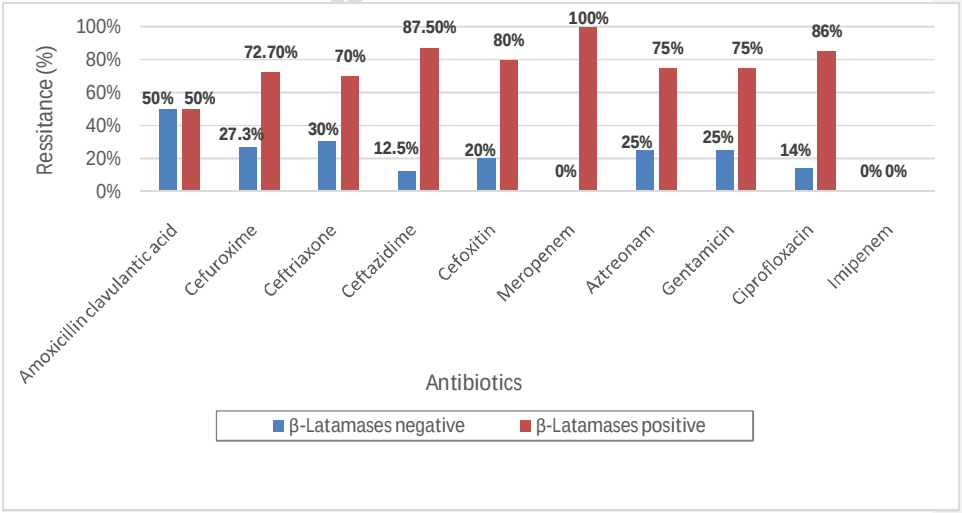

Fig. 1. Percentage of antibiotic resistance of beta-lactamase positive and negative strains

4. CONCLUSION

This study shows a high prevalence of β-lactamase-producing bacteria among patients admitted to the emergency unit. Again, β-lactamase producers were exclusively detected in patients coming from hospitals. Our finding is indicative of resistance still present at our local hospitals and less in our community. Also, there was a generally high resistance pattern of isolates to all the antibiotics used in this study. Therefore, control measures such as health education of the population in the field against self-medication, good cooperation between the clinician and the microbiologist as well as strict respect of hygiene measures are to be accentuated to eradicate the resistance of bacteria to antibiotics.

ETHICAL APPROVAL

The protocol of this study was approved by the Faculty of Health Science Institutional Review Board and the Yaoundé University Teaching Hospital (Number: 195U/AR/CHUY/DG/DGA/CAPRC). Participation was voluntary and each subject involved in the study gave written consent.

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