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Low Efficiency of the Commonly Prescribed Drugs against *Klebsiella pneumonia*, *Escherichia coli* and *Acinetobacter* Species as the Causative Agents of Blood Stream Infection in Malabo, Equatorial Guinea

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Abstract

The prevalence of multi drug resistant gram-negative bacteria to commonly first line drugs in blood is a serious problem in Equatorial Guinea and other world. This is the first study describing antibiotic resistance analysis of blood stream infection in Equatorial Guinea. Our study presents alarming rate of inefficiency of the most commonly prescribed drugs to treatment *Klebsiella pneumoniae*, *Escherichia coli* and *Acinetobacter* species isolates as the most frequency etiologic agents in blood stream infection. Out of 1849 blood culture the bacterial etiological agents were isolated from 196 (10.6%) samples. *E. coli* (n = 22), *K. pneumonia* (n = 39) and *Acinetobacter* (n = 17) represent 71.6% of all gram negative bacterial isolates. Almost all isolates of *K. pneumonia* and *Acinetobacter* sp. (92.1% and 100%, respectively) and about 50% of *E. coli* strains possessed extended-spectrum *β*-lactamase activity. Alarming level of multi drug resistant gram negative strains was observed. *E. coli* and *K. pneumonia* and *Acinetobacter* isolates demonstrated low sensitivity to all commonly prescribed drugs such as Ampicillin, Trimethoprim/Sulfamethoxazole, Doxycycline, Gentamycin Amoxicicline/Clavulanic Acid, Cefuroxime, Ciprofloxacine. It is especially worth noting the low efficiency of third generation cephalosporins (Cefrtiaxon) against Acinetobacter and Klebsiella with their resistance rate of 94.7% and 100% respectively. Moreover, the alarming level of low sensitivity to Piperacilin/Tazobactam of *K. pneumonia* (22%) and *Acinetobacter* (29.4%) was been found. The 17.6% of Acinetobacter isolated was carbomenem resistant. Just Imipenem and Amikacin were the most sensitive drug against these bacterial strains.

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Keywords

Bacteremia, Multi Drug Resistance, ESBL, Gram Negative Rods

1. Introduction

Bloodstream infections (BSIs) are among the most serious medical problems with high mortality and morbidity rates in the world and especially in Africa [1]-[4]. Gram-negative bacilli such as *Enterobacteriaceae* and non-fermenting gram-negative bacilli are the leading cause of bloodstream infection and responsible for the high mortality rates. Extended-Spectrum *β*-Lactamase (ESBL) producing strains are the most important problem as etiology of bacteremia [5]-[8]. The increasing incidence of infections due to antibiotic-resistant gram-negative bacilli in recent years is of great concern [9] [10]. Rapid diagnosis, identification of the causative agents of bacteremia and understanding resistance patterns may help guide effective empirical antibiotic therapy.

In Equatorial Guinea, the etiology of bloodstream infections is still not clear. The problem of antibiotic resistance is compounding because of overuse and misuse of antibiotics. We performed this prospective study in order to identify the most common causative agent of bacteremia and determine their antibiotic resistant pattern.

2. Subjects and Methods

The study was conducted at La Paz Medical Center from July 2013 to January 2016. La Paz Medical Center is a 150 beds hospital situated in the North of Bioko Island near the Malabo city, Equatorial Guinea.

During study period 1849 blood samples were obtained from patients admitted in different departments. These samples were quickly recorded and processed according to standard protocols for blood culture.

2.1. Samples Processing

The blood samples were inoculated into BD BACTEC Plus Aerobic/Anaerobic F medium and incubated in the BACTEC 9050 (Becton-Dickenson, USA) automat system at 35˚C for 5 days. Positive cultures were inoculated on Triptic Soy Agar with 5% sheep blood (TSAB), Mac Conkey's agar and Chocolate agar (HyLabs Ltd.) by spread plate technique [11]. The isolated bacteria were then identified by using Gram Staining procedure and their biochemical characteristics using Remelrap ID system kits (Rap ID ONE, Rap ID NF Plus).

2.2. Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined using the disc diffusion method on Mueller Hinton agar according to the Guidelines of the Clinical Laboratory Standards Institute (GCLSI) [12] [13].

Different families of antibiotics (discs obtained from OXOID, UK) were: Cephems (cefuroxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg)); Penicillins (Ampicillin (10 µg)); Beta-lactam + inhibitor (Amoxicillin/Clavulanic acid (20/10 µg), Piperacilin/Tazobactam (100/10 µg)); Aminoglycosides (Gentamicin (10 µg), Amikacin (30 µg)); Carbapenemes (Imipenem (10 µg)).

Confirmation of Extended-spectrum *β*-lactamase (ESBL) activity was performed by the standard double-disc synergy testing of Cefotaxime and Ceftazidime in the present and absent of Amoxicillin/Clavulanic acid.

Multi Drug Resistant bacteria are defined as resistant to at least three different classes of antibiotics [14].

2.3. Statistical Analysis

Statistical analysis was done using Microsoft Office Excel 2003.

3. Results

3.1. Epidemiology of Blood Stream Infection

During the study period, from July 2013 to January 2016 a total of 1849 blood cultures from different wards were collected: 417 (22.6%) from neonates, 623 (33.7%) from children and 783 (43.7%) from adult. The age of

patients was ranged from 0 year to 90 years. Bacterial or fungal pathogens were detected in 196 (10.6%) of cultures; of these 109 (55.6%) were Gram negative, 76 (38.8%) were Gram positive an 11 (5.6%) were yeasts (**Table 1**). For the Gram Negative rods, *Klebsiella pneumonia* was the most frequent isolate 39 (35.8%) followed by *Escherichia coli* 22 (20.2%), *Acinetobacter* species 17 (15.6%). These isolates represent 71.6% of all Gram Negative bacterial strains. Therefore, antibiotic resistance rates were performed specifically these three strains.

Coagulase-negative Staphylococci 49 (%) was the predominant Gram Positive cocci isolated from blood culture and was considered as probable contaminants from skin during the process of venipuncture. *Klebciella pneumonia* was the most causative agent of bacteremia in neonates, whereas *Acinetobacter* and *E. coli* were prevalent bacterial pathogens in bloodstream infection of adult patients. The prevalence of bacterial isolate in different age groups showed in **Table 2**.

3.2. Antibiotic Resistance

Very high level of resistance to the commonly used antimicrobial drug was observed. The comparison of antibiotic resistance/susceptibility rates was performed specifically for *E. coli*, *K. pneumoniae* and *Acinetobacter* sp. (**Tables 3-5**).

K. pneumoniae, *Acinetobacter* sp. and *E. coli* exhibited the highest percentage resistance to the Ampicillin (100%), Trimethoprim/Sulfamethoxazole (88.2%, 100%, and 88.9% respectively) and Doxycycline (90%, 66.7%, 82.4% respectively). More than 80% of *K. pneumoniae* and *Acinetobacter* sp. isolates were resistant to

Table 1. Prevalence of organisms isolated from blood cultures.

Pathogen	Total number (n = 196)
Gram negative bacteria n = 109 (55.6%)	
Klebsiella pneumonia	39 (19.9%)
Escherichia coli	22 (11.2%)
Acinetobacter sp.	17 (8.7%)
Pseudomonas aeruginosa	9 (4.6%)
Salmonella sp.	8 (4.1%)
Enterobacter sp.	5 (2.6%)
Burkelderia sepacia	4 (2.0%)
Serratia sp.	3 (1.5%)
Cirtobacter freundii	2 (1%)
Gram positive bacteria, n = 76 (38.8%)	
Coagulate negative staphylococci	47 (24%)
Staphylococcus aureus	11 (5.6%)
Streptococcus pneumoniae	4 (2.0%)
Streptococcus viridans group	4 (2.0%)
Streptococcus Group A	4 (2.0%)
Streptococcus Group D	4 (2.0%)
Rods gram positive	2 (1%)
Yeasts, n = 11 (5.6%)	

Table 2. The prevalence of *K. pneumoniae*, *E. coli* and *Acinetobacter* sp. in the different age groups of patients.

	K. pneumoniae	*E. coli*	*Acinetobacter* sp.
Neonates	18 (46.2%)	2 (9.1%)	3 (17.6%)
Children	8 (20.5%)	6 (27.3%)	4 (23.5%)
Adult	13 (33.3%)	14 (63.6%)	10 (58.9%)
Total strains	39 (100%)	22 (100%)	17 (100%)

Table 3. Antibiotic resistance rates for *K. pneumoniae* isolates from blood cultures.

Antibiotic subclass	Antibiotic	Total isolates	*Sensitive*		*Intermediate*		*Resistant*	
			n	%	n	%	n	%
Penicillins	Ampicillin	39	0	0.0	0	0.0	39	100.0
Beta-lactam + inhibitor	Amoxicillin/clavulanic acid	39	1	2.6	3	7.7	35	89.7
	Piperacilin/tazobactam	39	16	41.0	21	53.8	2	5.1
Cephalosporin II	Cefuroxime	38	1	2.6		0.0	37	97.4
Cephalosporin III	Ceftriaxone	38	1	2.6		0.0	37	97.4
Fluoroquinolones	Ciprofloxacin	30	4	13.3	6	20.0	20	66.7
Aminoglycosides	Gentamicin	39	5	12.8		0.0	34	87.2
	Amikacin	39	34	87.2	3	7.7	2	5.1
Tetracyclines	Doxycyline	10	1	10.0		0.0	9	90.0
Folate pathway inhibitors	Trimethoprim/ sulfamethoxazole	30		0.0		0.0	30	100.0
Carbopenemes	Imipinem	34	30	88.2	4	11.8		0.0

Table 4. Antibiotic resistance rates for *E. coli* isolates from blood cultures.

Antibiotic subclass	Antibiotic	Total isolates	*Sensitive*		*Intermediate*		*Resistant*	
			n	%	n	%	n	%
Penicillins	Ampicillin	22	0	0.0	0	0.0	22	100.0
Beta-lactam + inhibitor	Amoxicillin/clavulanic acid	22	3	13.6	6	27.3	13	59.1
	Piperacilin/tazobactam	22	16	72.7	3	13.6	3	13.6
Cephalosporin II	Cefuroxime	22	7	31.8	0	0.0	15	68.2
Cephalosporin III	Ceftriaxone	22	9	40.9	0	0.0	13	59.1
Fluoroquinolones	Ciprofloxacin	22	9	40.9	0	0.0	13	59.1
Aminoglycosides	Gentamicin	22	11	50.0	0	0.0	11	50.0
	Amikacin	22	19	86.4	3	13.6	0	0.0
Tetracyclines	Doxycyline	17	3	17.6	0	0.0	14	82.4
Folate pathway inhibitors	Trimethoprim/ sulfamethoxazole	18	2	11.1	0	0.0	16	88.9
Carbopenemes	Imipinem	22	22	100.0	0	0.0	0	0.0

Table 5. Antibiotic resistance rates for *Acinetobacter* sp. isolates from blood cultures.

Antibiotic subclass	Antibiotic	Total isolates	*Sensitive*		*Intermediate*		*Resistant*	
			n	%	n	%	n	%
Penicillins	Ampicillin	17	0	0.0	0	0.0	17	100.0
Beta-lactam + inhibitor	Amoxicillin/clavulanic acid	17	0	0.0	3	17.6	14	82.4
	Piperacilin/tazobactam	17	5	29.4	9	52.9	3	17.6
Cephalosporin II	Cefuroxime	17	0	0.0	0	0.0	17	100.0
Cephalosporin III	Ceftriaxone	17	0	0.0	0	0.0	17	100.0
Fluoroquinolones	Ciprofloxacin	17	5	29.4	1	5.9	11	64.7
Aminoglycosides	Gentamicin	17	6	35.3	0	0.0	11	64.7
	Amikacin	17	12	70.6	0	0.0	5	29.4
Tetracyclines	Doxycyline	9	3	33.3	0	0.0	6	66.7
Folate pathway inhibitors	Trimethoprim/ sulfamethoxazole	17	2	11.8	0	0.0	15	88.2
Carbopenemes	Imipinem	17	13	76.5	1	5.9	3	17.6

Amoxicillin/Clavulanic acid. About 60% of all isolates possessed resistance to fluoroquinolones (Ciprofloxacin). Alarming level of high rate resistant isolates of *K. pneumonia* (41%) and *Acinetobacter* sp. (29.4%) was found to Piperacilin/Tazobactam. 89.7% of *K. pneumoniae* isolates showed high rate of resistance to Gentamicin while *Acinetobacter* sp. and *E. coli* were 64.7% and 50.0% resistant respectively.

Among the antimicrobials testing, third generation cephalosporins (Cefrtiaxon) demonstrated the inefficiency against *Acinetobacter* and *K. pneumoniae* with their resistance rate of 94.7% and 100% respectively. The most active drug against *K.pneumoniae*, *E. coli* and *Acinetobacter* sp. was Imipenem, followed by Amikacin.

Based on their resistance pattern, the 100% of *K. pneumoniae* and *Acinetobacter*, 72.7% *of E. coli* strains were Multi Drug Resistant organisms. The 92.1% (n = 35) isolates of *K. pneumonia*, 100% (n = 17) of *Acinetobacter* sp. And 59.1% (n = 13) of *E. coli* were Extended-Spectrum *β*-Lactamase (ESBL) producing strains.

4. Discussion

According to the Antimicrobial Resistance Global Report of WHO the information and data about antibiotic resistance situation obtained from the most African countries is still not enough or clear [15]. Blood culture is still the gold standard for the diagnosis of bloodstream infections [2]. Rapid and correct choice of the antibiotic enables rapid cure of the patient and the saving of the patient's life.

In our study, out of 196 (10.6%) cultures were positive. This rate of positive samples from this study was lower than figures from previous studies [16]-[19] in Tanzania (13.4%), Central Africa (15.9%), Ethiopia (28%) and Zanzibar (14%). The difference in rates of positive samples can be explained by the differences in blood culture system, location and health situation in the region, capacity of hospital, epidemiological difference of the etiological agents [1] [3] [6] [16] [17] [20].

The antimicrobial resistance profile of *K. pneumoniae*, *E. coli* and *Acinetobacter* species isolates in our study revealed a generally higher resistance rate than reported in African studies [1] [16] [20]. The previous studies [1] [9] [21] [22] show the efficiency of Gentamicin against Gram Negative rods, but our data show a lower effectiveness of this antibiotic for treatment of *K. pneumoniae* and *Acinetobacter* isolates with their rate of resistance 87.2% and 64.7% respectively. For *E. coli*, *Acinetobacter* and *K. pneumoniae*, we reported high resistance (about 60%) to fluoroquinolones (ciprofloxacin) which limit the available oral treatment.

Multiple drug resistant Gram Negative bacteria are often associated with hospitals in developed countries where overuse/misuse of large amount of cephalosporin without special control. This antibiotic induces the formation of Extended-Spectrum *β*-Lactamase (ESBL) bacterial strains [23]-[25]. More over resistant organisms isolated during therapy to one cephalosporin may also demonstrate reduced susceptibility to other antibiotics not necessary the same chemical class [13] [26]. One explanation is spreading of antibiotic resistance by horizontal transfer of genetic material from one bacterial strain to an others. Antibiotic resistance genes may be transferred by different mechanisms of conjugation, transformation or transduction [9] [10] [27]. This lead to increase number of microorganisms with acquired resistance to second and third generation cephalosporins. [23] [26] [28] Our study have shown the highest level of resistance of *K. pnemoniae* (97.4%) and *Acinetobacter* sp. (100%) isolates to 3rd generation cephalosporins. Moreover, almost all isolates of *K. pneumonia* and *Acinetobacter* sp. (92.1% and 100% respectively) and about 50% of *E. coli* strains were Extended-Spectrum *β*-Lactamase (ESBL) producing strains. These results alarming and show how rapidly this phenomenon of Extended-Spectrum *β*-Lactamase (ESBL) strains spreads all over the world [7] [9] [15]. One way to decrease a high rate of Extended-Spectrum *β*-Lactamase (ESBL) producing bacteria is completely eliminate the use of cephalosporins as the first line drug and their replacement for a combination of Piperacillin or carbopenems with an aminoglycosides [7] [9] [26] [29]. Just Imipenem and Amikacin in our study possessed high efficiency against ESBL-producing gram negative bacteria such as *E. coli*, *K. pneumoniae* and *Acinetobacter* and may serve as prefered treatment. These results correlated with other studies in other part of the world [7] [9] [10] [15].

5. Conclusion

This is the first study describing antibiotic resistance analysis of blood stream infection in Equatorial Guinea. Our study shows alarming inefficiency of the commonly prescribed drugs against Extended-Spectrum *β*-Lactamase (ESBL) *E. coli*, *K. pneumonia* and *Acinetobacter* strains as causative agents of bacteremia. Just Imipenem followed by Amikacin was the most sensitive drugs.

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Conflict of Interest

The author declares that he has no competing interests.

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