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Evidence of the Transmission of Resistance of Enterobacteria to Betalactamines Between Mothers and Children in Brazzaville

Rachel Moyen[1, *], Saphia Jemylah Empilo Ndjiwa Galekoua[1], Jean Fabrice Yala[2], Bokatola Pea Indra Roenate[1], Tarcisse Baloki Ngoulou[1]

[1]Laboratory of Cellular and Molecular Biology, Faculty of Science and Technology, Marien Ngouabi University, Brazzaville, Congo
[2]Bacteriology Laboratory, Interdisciplinary Medical Research Center of Franceville (CIRMF), Franceville, Gabon

Email address:
rmoyen@yahoo.fr (R. Moyen)
[*]Corresponding author

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Abstract: Beta-lactams have always been used in many cases as first-line antibiotics in human medicine. The emergence of beta-lactamase-producing strains of Enterobacteriaceae is nowadays a public health problem. In order to demonstrate the transplacental transmission of enterobacteria resistance to beta-lactams between mothers and their children. Seventy-two (72) stool samples were collected from mothers and two-day-old newborns at the Talangaï referral hospital. Isolation of enterobacteria was done on selective media. Identification of beta-lactamase producing strains was performed by biochemical characteristics using API 20E strips. The resistance profile to carbapenems, monobactam and cephalosporins was performed by antibiogram. A total of 50 strains were purified and identified, of which 30 (60%) were isolated from the mother-newborn couple and 20 (40%) from the newborns. Among these strains, we identified in the mother-newborn group: 10 (33%) *Enterobacter* sp, 10 (33%) *Escherichia coli*, 6 (20%) *Klebsiella* oxytoca; 2 (7%) *Shigella* sp and 2 (7%) *Salmonella* sp. High resistance to carbapenems and cephalosporins was observed with *Shigella* sp. The other strains of enterobacteria isolated from newborns only, among which were identified: 7 (35%) *Escherichia coli*; 5 (25%) Klebsiella oxytoca; 5 (25%) *Enterobacter* sp, 3 (15%) *Shigella* sp. newborns without their mothers showed a variable resistance profile to the antibiotics tested. Three resistance phenotypes were observed in the mother-newborn group including: FEP PRL CL, FEP AMP MA CR ATM IMP ETP and FEP AMP CAZ MA CL ETP. The resistance phenotypes observed in the mothers were identical to those found in their respective offspring. These results show that each newborn is born with a rate of resistance acquired at birth, which testifies to a transplacental transmission between mother and child, consequence of the emergence of the resistance of enterobacteria to betalactamines in the Congolese population.

Keywords: Bacteria, Transmission, Neonatal, Antibiotics, Resistance

1. Introduction

The discovery and use of antibiotics since the end of the last 60 years have had a considerable impact on population health, medical practice and scientific research. The use of antibiotics has played a crucial role in the control of many infections and their development has revolutionized the treatment of infectious diseases. As a result, their discovery has been one of the main reasons for the dramatic increase in average life expectancy during the 21st century. Their importance is of great utility in public health [1]. However, with the increasing and sometimes unjustified use of these molecules, bacteria have learned to defend themselves and adapt. This adaptation gives them antibiotic resistance, which is one of the greatest threats to global public health [2]. It is estimated that 700,000 people die annually from infections due to antibiotic resistant bacteria [3]. Antibiotic resistance is

a natural phenomenon, but is accelerated by inappropriate antibiotic prescribing, poor infection control practices in humans, and inappropriate antibiotic use [4]. The direct consequence of this resistance is increased morbidity and mortality from bacterial infectious diseases [5]. This is the case for diarrhea, which is the second leading cause of child mortality [6]. A first approach in the fight against bacterial resistance involves prevalence studies of multi-resistant strains. Antibiotic resistance in bacteria has become a worrying problem today, as some diseases become more difficult to treat. This could lead to complications in children who have them. Treatment can also become more complicated. This is because many germs have developed resistance to β-lactams, which are the most commonly prescribed antibiotics. In newborns, who have not received any antibiotic therapy, the microbiota, still wild, should be sensitive to antibiotics for which no natural resistance has been described. The emergence of antibiotic resistance observed may be due to the acquisition of resistance genes through transplacental transmission from mother to child [7].

In Congo, no studies have been conducted in this context, although antibiotic resistance rates in community and hospital-acquired bacteria are very high.

It is with this in mind that this study aims to highlight the transplacental transmission of beta-lactam resistance for effective management of newborns with bacterial infections due to enterobacteria.

2. Materials and Methods

2.1. Sampling, Inclusion Criteria

Sampling was done in the maternity ward in two groups: one group consisting of 30 samples from mother-newborn couples and one group consisting of 20 samples from newborns only, for a total of 50 samples. Anal swabbing was performed.

The samples were packed in sterile freezer bags, placed in a cooler containing cold accumulators and quickly transported to the laboratory. Only neonates in the maternity ward who had not received any antibiotic treatment and whose age was between 0 and 2 days were included in this study. Their enrollment was made possible by the agreement of their mothers who read and approved the informed consent. For illiterate mothers, the consent was read and translated in the presence of the head of the maternity ward.

2.2. Microbiological Analysis

2.2.1. Isolation

Inoculation was done on five culture media: *Salmonella - Shigella* agar for the isolation of *Salmonella* and *Shigella* bacteria, Methylene Eosin Blue (EMB) for the isolation of Enterobacteriaceae, as well as Cled, Mac Conkey and Bile Esculin and Sodium Azide (BEA) media for the isolation of Enterobacteriaceae. The plates containing the different media were inoculated with the cotton swab tip using the 3-quadranttechnique. The plates were then incubated at 37°C for 18 to 24 hours.

2.2.2. Purification of Strains

Purification was performed in nutrient agar. Each colony was streaked separately until a distinct and homogeneous colony was obtained. To ensure the purity of the strains, microscopic observation was performed. The characterization of the isolates started with the application of classical microbiology techniques, based on the research of a certain number of characters (cell morphology by the fresh state under the optical microscope.

2.2.3. Identification of Strains

The API 20E gallery was used for the identification of enterobacteria.

2.3. Determination of the Antibiotic Resistance of the Isolated Bacteria

This was carried out by standard antibiogram by diffusion in agar medium. The inoculum (bacterial suspension) was prepared by emulsifying three colonies of an 18-hour-old culture in 5 mL of sterile physiological water. The bacterial suspension was then well homogenized. Its opacity was equivalent to an OD of 0.08 to 0.1 read at 625 nm [8] corresponding to the 0.5 Mac Farland value. This is equivalent to a pure barely confluent culture of 10^6 to 10^8 CFU/ml (CA-SFM, 2020).

The inoculum was seeded on Mueller-Hinton medium poured into a Petri dish uniformly with a thickness of 4mm [9]. The following antibiotics were applied on Mueller Hinton medium: piperacillin (PRL), cephalexin (CL), cefepime (FEP), ampicillin (AMP), ceftazidine (CAZ), cefamandole (Ma), cefpirone (CR), aztreonam (AZM), mipenem (IMP) and ertapenem (ETP). Plates were inoculated by swabbing and incubated at 37°C for 24 hours followed by reading and interpretation according to CA-SFM (2020).

2.4. Determination of Antibiotic Resistance Phenotypes of Isolated Bacteria

The determination of the resistance phenotypes of the antibiotics to which the bacteria show resistance was done from the results of the standard antibiogram. Thus, for each bacterium, the resistance phenotype was written literally using the symbols of the tested antibiotics.

3. Results

3.1. Isolation

A total of 72 bacteria were isolated. The distribution of results by group was as follows:

1. Mother-newborn group: 46 bacteria of which 23 isolates were from the mothers' samples and 23 isolates from their newborns' samples, for a total percentage of 64%.
2. Newborn group only: 26 isolates with a percentage of 36% (Figure 1).

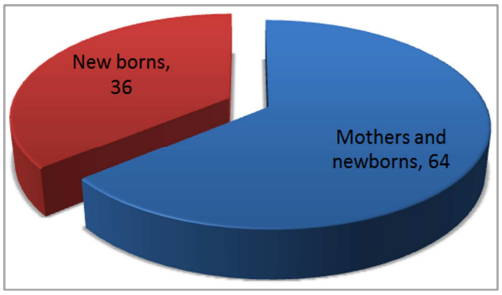

Figure 1. *Distribution of isolated bacteria.*

3.2. Bacterial Identification

Microscopic observation allowed to determine that among the isolated strains, 80% are immobile while 20% are mobile. These cells are mostly arranged in isolated rods (70%) and in bacilli chain (30%).

Gram staining and catalase test revealed that all strains were Gram negative and only 80% were catalase positive.

Table 1. *Identification of strains in the mother-newborn group.*

Type	Couple mothers and newborns		
	Mothers	Newborns	Pourcentages
Enterobacter sp.	5	5	33%
Escherichia coli	5	5	33%
Klebsiella oxytoca	3	3	20%
Salmonella sp.	1	1	7%
Shigella sp.	1	1	7%
Total	30		100%

The API 20E gallery allowed the identification of 30 strains in the mother-newborn couple group, distributed as follows 10 *Enterobacter* sp (33%), 10 (33%) *Escherichia coli*, 6 *Klebsiella oxytoca* (20%); 2 *Shigella* sp (7%) and 2 *Salmonella* sp (7%) (Table 1).

Concerning the newborn group only, the API 20E gallery identified 20 strains including: 7 (35%) *Escherichia coli*; 5 (25%) *Klebsiella oxytoca*; 5 (25%) *Enterobacter sp*, 3 (15%) *Shigella* sp. (Figure 2).

Figure 3 illustrates the identification of strains in the mother-newborn pair and neonate groups only. It shows that *Enterobacter sp* and *Escherichia coli* are the most predominant bacterial strains in the mother-newborn group, but *Salmonella* sp. was not isolated and identified in this group.

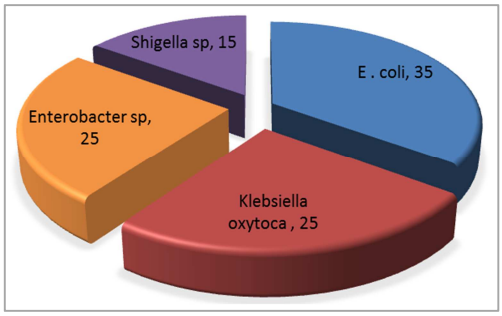

Figure 2. *Identification of strains in the newborn group only.*

Legend: FEP: Cefepime; PRL: Piperacillin, AMP: Ampicillin; Caz: Ceftazidine; Ma: Cefamandole; CR: Cefpirone; ATM: Aztreonam; CL: Cephalexin; IMP: Imipenem; ETP: Ertapenem.

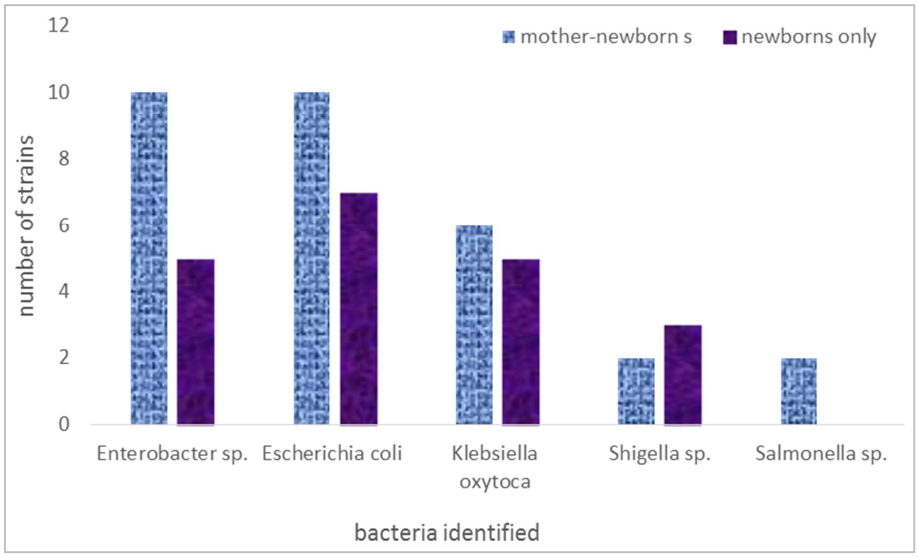

Figure 3. *Identification of strains isolated from both groups.*

3.3. Determination of Antibiotic Resistance

This determination of antibiotic resistance was done in consideration of the two groups.

3.3.1. Case of Group 1: Mother and Newborn Pair

Ten (10) antibiotics of the beta-lactam family were tested on four (4) strains of Klebsiella oxytoca. Figure 4 shows that all strains were 100% resistant to piperacillin, cephalexin and cefepime. The other molecules (ampicillin, ceftazidine, cefamandole, cefpirone, aztreonam, imipenem and ertapenem) have good positive activity on these four (4) strains of Klebsiella oxytoca. *Enterobacter* sp. strains were all 100% resistant to cefepime, ampicillin, cefamandole and ertapenem.

However, these (4) strains were 100% sensitive to piperacillin.

In *Escherichia coli*, the strains showed no resistance to piperacillin and ertapenem. All the *Escherichia coli* strains presented various percentages of resistance which are the following: 100% of resistance to ampicillin, cefamandole and cefalexin. 50% to cefepime, ceftazidine, aztreonam and imipenem.

Concerning *Shigella* sp. strains, ceftazidine, cefpirone, aztreonam and imipenem had a good activity on the tested *Shigella* sp. strains. In contrast, all remaining molecules had 100% resistance to the 2 *Shigella* sp. strains. As shown in Figure 5.

The analysis of the results of the antibiotic resistance test showed that the *Salmonella* sp. strains were resistant to two selected antibiotics belonging to the β-lactam family. These were cefepime and cefamandole.

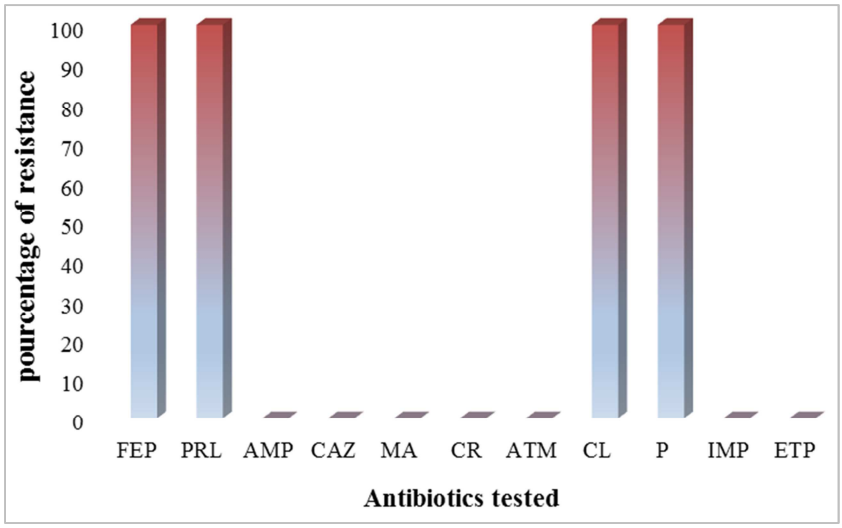

Figure 4. *Resistance profile of 4 strains of Klebsiella oxytoca.*

Legend: FEP: Cefepime; PRL: Piperacillin, AMP: Ampicillin; Caz: Ceftazidine; Ma: Cefamandole; CR: Cefpirone; ATM: Aztreonam; CL: Cephalexin; IMP: Imipenem; ETP: Ertapenem.

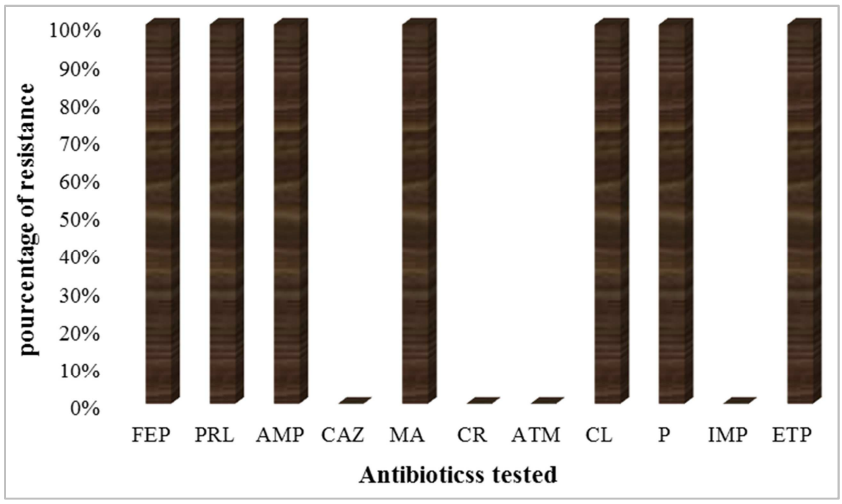

Figure 5. *Resistance profile of the two (2) strains of Shigella sp.*

Légende: FEP: Céfepime; PRL: Pipéracilline, AMP: Ampicilline; Caz: Céftazidine; Ma: Céfamandole; CR: Cefpirone; ATM: Aztréonam; CL: Céphalexine; IMP: Imipénème; ETP: Ertapénème.

3.3.2. Group 2 Cases: Neonates Only

Of all the strains identified from the newborn only group, 12 strains including: 3 strains of Klebsiella oxytoca, 3 strains of *Enterobacter* sp., 3 strains of *Escherichia coli*, and 3 strains of *Shigella* sp. were found to be resistant to antibiotics.

The results represented in figure 6 below show us the resistance profile of the different strains tested. All strains showed 100% resistance to ampicillin. *Shigella* sp was 33% resistant to carbapenems (imipenem and ertapenem). Only cefpirone had good activity on all these strains. On the other hand, *Shigella*sp. was resistant to cefpirone, i.e., a 33% resistance rate.

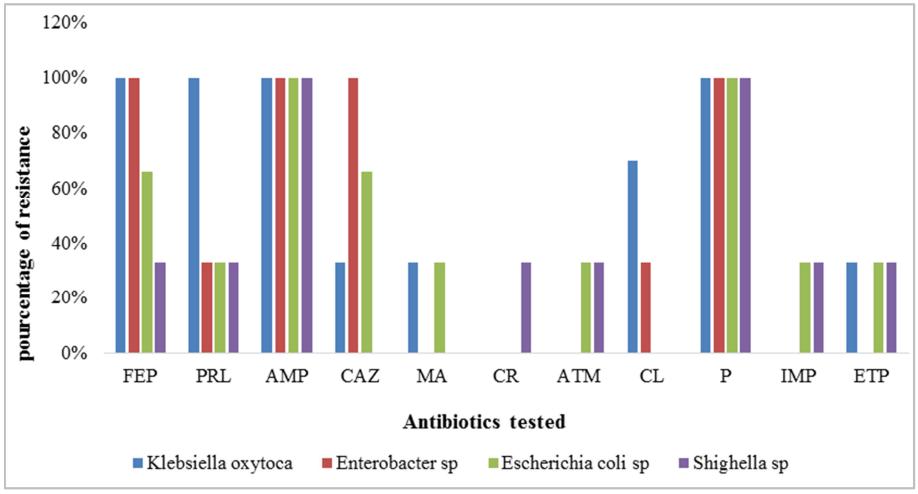

Figure 6. *Resistance profile os strains of: Klebsiellaoxytoca, Enterobacter sp., Escherichia coli et de Shigella sp.*

Legend: FEP: Cefepime; PRL: Piperacillin, AMP: Ampicillin; Caz: Ceftazidine; Ma: Cefamandole; CR: Cefpirone; ATM: Aztreonam; CL: Cephalexin; IMP: Imipenem; ETP: Ertapenem.

3.4. Determination of Antibiotic Resistance Phenotypes of Isolated Bacteria

The results of the antibiotic resistance phenotypes of Klebsiella oxytoca strains are shown in Table 2. Indeed, all four strains had the same antibiotic resistance phenotype including FEP PRL CL. Our results also show that for the *Enterobacter* sp. strains S5 and M5 from the newborn and its respective mother, present the same resistance phenotype that is FEP AMP MA CR ATM IMP ETP with an appearance rate of 50%; while for the strains S11 and M11, the resistance phenotype is: FEP AMP CAZ MA CL ETP, with a percentage of 50% (Table 3).

Table 2. *Antibiotic resistance phenotypes of Klebsiella oxytoca strains.*

Strains	Observed phénotypes			Number of appearances	Pourcentage
S1	FEP	PRL	CL	1	25%
M1	FEP	PRL	CL	1	25%
S4	FEP	PRL	CL	1	25%
M4	FEP	PRL	CL	1	25%

Legend: S: strain from the newborn; M: strain from the mother.

Table 3. *Antibiotic resistance phenotypes of Enterobacter sp.*

Strains	Observed phénotypes									Pourcentage
S5	FEP	AMP		MA	CR	ATM		IMP	ETP	25%
M5	FEP	AMP		MA	CR	ATM		IMP	ETP	25%
S11	FEP	AMP	CAZ	MA			CL		ETP	25%
M11	FEP	AMP	CAZ	MA			CL		ETP	25%

Legend: S: strain from the newborn; M: strain from the mother.

4. Discussion

In this study, carried out on a group of 30 samples from mother-newborn couples and a group of 20 samples from newborns only, the search for microorganisms (enterobacteria) in the faeces of these target groups of our study allowed us to isolate 72 isolates on the different media used, 64% of which were from mother-newborn couples and 36% from newborns only. The results of our study showed that the germs isolated were Klebsiella oxytoca, *Enterobacter* sp., *Escherichia coli* and *Shigella* sp. [2], in a similar study on the epidemiology and mother-to-child transmission of extended-spectrum beta-lactamase-producing Enterobacteriaceae (E-BLSE) in Madagascar also found Enterobacteriaceae in mothers and children. The results of his study highlighted the presence of the isolated germs, *Enterobacter* sp in particular. Thus, our results are in agreement with the results obtained in this study. The incidence of these bacterial infections could have a negative impact on the health of newborns.

The bacterial isolates showed various cultural aspects, depending on the culture medium, allowing us to suspect their belonging to a bacterial group (enterobacteria). The Gram test determined that all isolates were Gram negative according to the medium used. Our results can also be paralleled with those of Bouguenoun [10] that he obtained in

his study conducted on the antibiotic resistance of bacteria incriminated in nosocomial infections caused by the presence of enterobacteria including BGN predominantly. Indeed, this author reported that in nosocomial bacterial infections caused by the presence of enterobacteria, Gram-negative bacteria were present in majority.

The catalase test confirmed that these isolates belonged to the enterobacteria family. This test was positive for 80% of all isolates tested. If we compare our results with those found by Welton et al. [11], in their work on the catalase test as an aid to the identification of enterobacteria, these results corroborate ours.

The Api 20E gallery was used to identify the different enterobacteria. As a result, the most isolated species are in decreasing order: *Escherichia coli* (36%), *Enterobacter* sp. (30%), *Klebsiella oxytoca* (22%), *Shigella* sp (5%) and *Salmonella* sp (4%). These same species were isolated both from newborns and from mothers and their newborns (mother-newborn pair group). As can be seen, *Escherichia coli* is the most isolated enterobacteria, presumably because of its ability to adhere to cells, followed by *Klebsiella pneumoniae* as reported by Kone et al. [12] and Tondji et al. [13]. It is the most encountered bacterium in these two identified groups. The presence of these enterobacteria could be due to contaminations following bacterial transmission from the mother to her newborn which could be transplacental. This transmission could have occurred during pregnancy, labor, or delivery. Indeed, Peretz et al. [14] in his work found that, some neonates acquire ESBL bacteria from their mothers during labor. Bonfanti et al. [15] related mother-to-child transmission particularly of carbapenemase producing KPC *Klebsiella Pneumoniae* at birth. Similarly, Krämer et al. [16] in his research demonstrated transmission of *Salmonella typhimurium* from a mother to her newborn twins. In the same vein, Parisot et al. [17] observed in a study focused on Shigellosis and pregnancy in French Guiana: obstetric and neonatal complications, three cases of mother-to-child transmission of *Shigella*.

It has also been shown that the transmission of certain strains of enterobacteria could also occur during breastfeeding. Indeed, Salah et al. [18] showed that human breast milk served as a source of transmission of *Salmonella enterica* serotype Typhimurium from a mother to newborn consanguineous twins.

Determination of antibiotic resistance of the strains isolated in the mother-newborn group showed that all the bacteria studied showed fairly high resistance to almost all the beta-lactam family. The high prevalence of ESBL-producing Enterobacteriaceae goes hand in hand with a very high prevalence of antibiotic-resistant Enterobacteriaceae. Cecile et al. [19] made the same observation in a study evaluating antibiotic resistance in enterobacteria isolated from patients in a hospital in Douala, Cameroon. This antibiotic resistance would be consecutive to the inappropriate and exaggerated use of these molecules by mothers in treatment.

Concerning *Klebsiella oxytoca*, relatively high rates of resistance have been observed with piperacillin, cephalexin and cefepime molecules. This indicates that strains of these bacteria would have produced penicillinases and beta-lactamases, thus conferring resistance to first generation cephalosporins. These relatively high rates of resistance to piperacillin, cephalexin and cefepime justify that these molecules are no longer recommended for probabilistic treatment of these bacterial infections. However, our results are lower than those of Sekhri et al. [20], who found resistance rates of 43.35% to ampicillin and 73.07% to cefazolin respectively. In consideration of the above, this acquired resistance is thought to be related to antibiotic abuse [21], resulting from hyperproduction of penicillinase [22].

Carbapenems had good activity on *Klebsiella oxytoca*. These results are consistent with the results obtained by Nouri et al. [23] on the bacteriological and antibiotic resistance study of *Klebsiella*. This author had found a rate of 80% of *Enterobacter* sp. This bacterium developed resistance to carbapenems including ertapenem, a molecule of choice, at a rate of 100%, as well as cefepime and ampicillin in the same proportion as well, i.e., 100%. *Enterobacter sp. also* showed varied resistance to the 3rd and 4th generation cephalosporins tested. On the other hand, Gadou et al. [24] had found much lower results which showed that *Enterobacter* sp strains were sensitive to imipenem but 33.3% resistant to ertapenem.

Antibiotics tested on the different *Escherichia coli* strains showed varying percentages of resistance: The resistance profile shows 100% resistance to ampicillin, cefamandole, cefalexin; 50% to cefepime, ceftazidine to aztreonam and imipenem. Only ertapenem had 100% positive activity on *Escherichia coli.* This suggests overuse of these broad-spectrum antibiotics. These strains would produce carbapenemases and broad spectrum betalactamases causing enzymatic resistance, which would hydrolyze these tested antibiotic molecules. Nevertheless, in this same register, studies conducted by Gadou et al. [24] showed that *Escherichia coli* strains were sensitive to imipenem but these same strains were 27.3% resistant to ertapenem. Obviously, this rate is still low compared to the rates reported above.

For *Shigella* sp. ceftazidine, cefpirone, aztreonam and imipenem had good activity on both strains of *Shigella* sp. These strains showed 100% resistance to cefepime, piperacillin, ampicillin, cefamandole, cefpirone and ertapenem. This result corroborates those of Aboubacar et al. [25] who reported the 100% sensitivity of imipenem and ceftazidine molecules on the majority of *Shigella* sp strains, following a study on antibiotic resistance in enterobacteria isolated in Bamako, Mali.

In *Salmonella*sp, all the strains were 100% sensitive to the tested penicillins and carbapenem, the resistance was observed for cephalosporins as to cefepime and cefamandole. This suggested that these strains have low cephalosporinase activity.

These results are consistent with that of Mitima et al. [26] who obtained a proportionate 81.7% positive activity rate of ceftazidime on *Salmonella* spp. These results are also similar to those of Threlfall et al. [27] who observed very low resistance of *Salmonella* spp to third generation

cephalosporins. The resistance rate was around 0.6% only.

Finally, the study of antibiotic resistance of strains isolated in the neonate group only reveals that all strains showed 100% resistance and to ampicillin. *Shigella* sp had 33% resistance to carbapenems (imipenem and ertapenem). Since these neonates never received antibiotic treatment immediately after birth, it would be assumed that there was a transfer of antibiotic resistant strains from mothers to neonates. This assertion would be proven.

Regarding the resistance phenotypes, it is observed that the randomly selected strains show the same resistance phenotype in the mother and newborn pair. These results indicate that there is transmission of resistance from mother to newborn, since these newborns have never been exposed to antibiotic treatment.

In sum, it should be noted that the levels of resistance obtained in our study are worrying and alarming. This situation is the consequence of the excessive prescription and sometimes abusive use of broad-spectrum antibiotics in both hospital and community settings.

5. Conclusion

Our study focused on the prevalence of extended-spectrum Betalactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae in newborns at the Talangaï referral hospital in Brazzaville. The production of ESBL was the resistance phenotype sought in BGN. This study gave us an overview of the prevalence (frequency) of enterobacteria in mothers and newborns. It was found that *Escherichia coli* and *Enterobacter* sp were the most frequently isolated Enterobacteriaceae strains and their enzymatic resistance activity was evident to varying degrees. Indeed, the resistance study showed that most of the bacterial species had a very high resistance to the cephalexin tested. However, we found high rates of 63% and 50% of resistance of the *Enterobacter* sp strains isolated against imipenem and ertapenem respectively. Similarly, *Klebsiella oxytoca* had high rates of resistance to piperacillin, cephalexin and cefepime. Finally, *Salmonella* sp, was 100% susceptible to the antibiotics tested, except cefepime and cefamandole.

This high rate draws attention to the frequency of bacterial resistance in our country. Particular attention must be paid to the respect of the rules for the use of antibiotics and to the need for increased and rigorous surveillance of mothers and newborns.

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