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Original Research Article

Multiresistant Bacteria in Neonatal Intensive Care and the Contribution of Molecular Biology

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Abstract

Epigenetics is a very active field of research today. This is a mechanism by which environmental factors can influence gene expression. Indeed, epigenetics has been defined as a new science aimed at studying the mechanisms by which the genotype generates the phenotype, without there being any modifications in the DNA sequence. In this context, we conducted a prospective study on the records of 523 patients hospitalized in the neonatal intensive care unit of the CHU Mohamed VI in Marrakech, over a period of 6 months, from July 01, 2019 to December 31, 2019. Main results obtained were as follows: A high MRB carriage rate affecting 45.5% of hospitalized patients during the study period. Late MRB infections are an important part of this. The analysis of the risk factors of these infections had demonstrated the major role of invasive procedures, in particular mechanical ventilation and KTVO, as well as the high duration of hospitalization which was on average 17.47 days in our study. The bacteriological profile was dominated by enterobacteriaceae, particularly K. pneumoniae and E. cloacae. Of all the MRBs sent to the Kremlin Bicêtre hospital, 55 strains were analyzed by MLST; including 36 strains of K. pneumonia and 19 strains of E. cloacae. The Enterobacteriaceae strains analyzed in our study were mainly carbapenemase producers, of the NDM and OXA-48 type, and ESBL type CTX-15M. Of all the bacterial clones identified, a high rate of resistance to antibiotics, in particular carbapenems, was noted in the following clones: ST 1805, ST 1158 and ST 307. The ST 1805 clone was exclusively found in our series.

Keywords: Epigenetics; Nosocomial infection; Multi-resistant bacteria; Molecular biology.

1. INTRODUCTION

The U.S. Department of Health and Human Services Centers for Disease Control and Prevention defines nosocomial infection as an infection during hospitalization that was not present or was incubating at the time of admission. This is the equivalent of a late infection which occurs after 72 hours of life [1]. Newborns hospitalized in a neonatal intensive care unit are at high risk of developing a nosocomial infection due to immune immaturity and invasive diagnostic and therapeutic procedures [2]. Nosocomial sepsis (which is essentially bacteremia) should be distinguished from nosocomial infection (which can affect several organs).

The use of antibiotics has considerably reduced mortality and morbidity linked to bacterial infections. Nevertheless, the irrational use of antibiotics has led to the emergence of an alarming number of resistances. Recent research points to the role of epigenetics in the development of these resistances.

Over the years, the literature has focused mainly on the study of the genetic bases of bacterial resistance to antibiotics. However, some studies suggest that bacterial genetics alone cannot explain the rapid development of this resistance or its reversibility (especially in adaptive resistance). Indeed, bacterial epigenetics can provide new answers in this regard [3].

Epigenetics is thus redefined as being the study of modifications in the activity of genes which are hereditary from a mitotic and/or meiotic point of view, not involving a modification in the DNA sequence. Unlike mutations, epigenetic modifications are reversible [4].

In intensive care, the fight against MRB is part of the policy for the prevention of nosocomial infections. The control of cross-transmission and the reduction of selection pressure, through rational use of ATBs, are the two essential components.

The objective of this study was to determine the incidence of multi-resistant bacteria in neonatal intensive care by analyzing their clinical, therapeutic and evolutionary impact and to determine Beta-lactam resistance genes and the clonal diversity of muti-resistant bacteria responsible for neonatal bacteraemia in a study conducted in the neonatal intensive care unit of the CHU Mohamed VI in Marrakech.

2. MATERIALS AND METHODS

2.1. Study population:

This study focused on a cohort of patients admitted to the neonatal intensive care unit of the Mohamed VI University Hospital in Marrakech over a period of six months, from July 1 to December 31, 2019 and staying more than 48 hours in intensive care. Our service is made up of 13 rooms with a capacity of 22 incubators, 14 of which are reserved for intensive care and 8 reserved for neonatal care. We attach great importance to hygiene measures in the service. In our study, all newborns hospitalized during this period were included. Among these records were selected those that meet the definition of infection (diagnosis based on a range of clinical, biological, radiological and microbiological arguments).

They were excluded from the study, the files of the patients having not presented a bacterial infection. Of the total of 523 hospitalizations during the study period, 686 blood cultures were performed in patients hospitalized in the neonatal intensive care unit of the CHU Mohamed VI in Marrakech.

The bacterial identification of MRB at the level of the bacteriology laboratory of the CHU Mohamed VI in Marrakech was based on conventional morphological, cultural, biochemical and antigenic characters.

Of all the MRB s isolated, 70 strains were sent to the Kremlin Bicêtre Hospital in Paris, 55 of which were analyzed.

In this study, the MRB isolates analyzed were treated by the MLST (MultiLocus Sequencing Typing) technique in order to determine the beta-lactam resistance genes.

Statistical analysis of the data was carried out using SPSS version 23 software. The results were expressed as percentages, or as means depending on the variables studied.

3. RESULTS

3.1. Characteristics of the population studied

During this study period, the neonatology and neonatal intensive care unit in Marrakech experienced the hospitalization of 523 newborns. They totaled 4505 days of hospitalization, with an average duration of 8.61 days (+/- 0.354). The average weight was 2461.73 g (from 700 g to 5200 g) and the average admission age was 4.92 days. The average gestational age was 36.79 SA +/- 3.5 weeks, with a percentage of 31.73% of infected premature babies. Admission pathologies were dominated by neonatal infection (isolated in 16.3% of cases and associated with other pathologies in 83.7% of cases) (Table I).

3.2. Characteristics of nosocomial bacterial infections:

In the study population; 54.50% presented a nosocomial infection with multiresistant bacteria, of which 73.20% represented late infections. With an average duration of hospitalization of late MRB bacteremia was 17.47 days against 8.68 days for early MRB bacteremia.

The average gestational age of newborns who had presented a late MRB infection was 36.18 weeks +/- 2.4 weeks with extremes ranging from 28.3 weeks to 41.4 weeks (with a rate of prematurity from 55% to 28.50% predominated by late infections). Of the 60 patients with late MRB infection, 40 were male (66.4%) with a sex ratio of 2. The average weight of neonates with late MRB infection was 2172 g (ranging from 900g to 4800g). The average duration of mechanical ventilation was 12.4 days in late MRB infections with a percentage of 90%. The average duration of KTVO was 7.9 days with a percentage of 33.4% in patients who presented with late MRB infection.

The bacteriological profile of late MRB infections in our context was predominated by enterobacteriaceae, particularly K. pneumoniae and E. cloacae; which represented respectively 51.42% and 37.15%. The other MRBs, namely K. oxytoca, P. oryzihabitans, ABMR and PAMR, were found at a lower frequency.

Of the 60 patients who had a late MRB infection, 31 had progressed favorably, i.e. 51.6% of cases, against 29 who died during their hospitalization, i.e. 48.7% of cases.

Concerning early MRB bacteraemia, the favorable evolution was noted in 45.4% of cases, against a mortality rate of 54.6%.

3.3: Comparison of newborns infected with MRB:

The comparison is illustrated below (table 2), this comparison shows that the average length of stay of patients who presented with a late MRB infection was

greater than that of early infections (17.47 +/- 15 versus 8.86 +/- 7) with a p=0;001 (highly significant).

The use of KTVO and ventilation is more frequent in the late infections with a significant result (p=0,017 for KTVO and p=0,001 for ventilation). The longer the duration of ventilation, the more likely it is to cause late infections with a very significant result (p=0,006).

3.4. Results of bacterial clones associated with Enterobacteriaceae:

In our study, 66% of the analyzed strains of K. pneumonia (Figure 1) produced at least one carbapenemase. Of these, 38% produced an NDM-type carbapenemase (36% for NDM-1 and 2% for NDM-7), 25% produced an OXA-48-type carbapenemase and 3% co-produced 2 types of carbapenemases: NDM-7 and OXA-48. The ESBL production rate was 34% of cases.

The sequencing of the genome of these strains by MLST technique, made it possible to identify 10 different bacterial clones (Figure 2), with predominance of clone ST 1805 (32%), followed by ST 307 (20%) and then ST 25 (11%). ST 1805 and ST 478 clones were associated with NDM production, ST 327 was associated with OXA-48 production, and ST 25 and ST 307 were associated with ESBL production.

Regarding the strains of E. cloacae analyzed in our study (Figure 3), 60% produced at least one carbapenemase. The distribution of carbapenemases found was as follows: overwhelmingly OXA-48 (25%), then NDM-1 (20%), followed by NDM-7+OXA-48 (15%). The ESBL type CTX-M15 production rate was 35%.

As for the analyzed strains of E. cloacae the sequencing of their genomes also made it possible to identify 10 different bacterial clones (Figure 4), with the predominance of clone ST 344 (21%), followed by ST 1158, ST 110 and ST66 (11%). ST 344 was associated with the production of OXA-48, ST 1158 was associated with the production of both OXA-48 and NDM, ST 110 was associated with the production of ESBL and NDM, and ST 66 was associated with the production of an NDM-1.

3.5. Distribution of bacterial clones according to risk factors:

Of all the bacterial clones analyzed, 88.5% were associated with mechanical ventilation. In our series, 25.7% of the bacterial clones analyzed were associated with the use of a KTVO.

Prematurity was found in 62.8% of the identified bacterial clones. 45.7% of the bacterial clones analyzed were associated with IUGR (Table 3).

A high death rate was present especially at the clone level: 147, 307, 344, 478. The highest rates of resistance to antibiotics, in particular to carbapenems in the enterobacteria analyzed, were observed in the following bacterial clones: ST 1805, ST 1158 and ST 307.

Annexes

Table I: Comparison of newborns infected with MRB

Diagnostic	Newborns	Newborns infected with IBNN*	Newborns not infected with IBNN*
Viral bronchiolitis	6	1	5
Choanalatresia	2	1	1
Congenitalheartdisease	69	26	43
Polymalformatif syndrome	6	2	4
Retroviral infection	4	3	1
Polycystickidneydisease	1	0	1
Pneumothorax	2	1	1
Malfomativeuropathy	2	0	2
Congenitalichtyosis	1	0	1
Others	345	110	235
Total	**438**	**144**	**294**

** IBNN: neonatal nosocomial bacterial infection*

Table 2: Comparison of newborns infected with MRB

	Total infected patients (n=82)	Early MRB infection (n=22)	Late MRB infection (n=60)	OR (95%)	p-value
Male (%)	51	11 (50%)	40 (66%)		
Feminine (%)	31	11 (50%)	20 (44%)		
sex ratio	1.6	1	2		
Average age in days	3.06	5.32	2.23	1.77 – 4.35	0.297

	Total infected patients (n=82)	Early MRB infection (n=22)	Late MRB infection (n=60)	OR (95%)	p-value
(standard deviation)					
Gestationalage (WA)					
<35	30 (36.58%)	11 (50%)	19 (31.6%)		
35-37	14 (17.07%)	3 (13.63%)	11 (18.33%)		
>=37	38 (46.34%)	8 (36.36%)	30 (50%)		
Medium	35.79 +/- 3.65	34.71 +/- 3.48	36.18 +/- 3.66		0.052
Admission weight:					
1000 - 1499g	25 (30.48%)	7 (31.8%)	18 (30%)		
1500 - 1999g	23 (28.04%)	7 (31.8%)	16 (26.6%)		
2000 - 2499g	8 (9.75%)	4 (18.18%)	4 (6.6%)		
2500 - 3999g	23 (28.04%)	4 (18.18%)	19 (31.6%)		
> 4000g	3 (3.65%)	0	3 (5%)		
Medium	2056.77g +/- 864	1864.09 +/- 646	2127.42 +/- 926	1866.8 – 2246.74	0.063
Average length of stay (days)	16.16 +/- 13	8.86 +/- 7	17.47 +/- 15		0.001
KTVO (%)	27 (32.9%)	7 (31.8%)	20 (33.3%)	0.933 (0.328 – 2.655)	0.017
Average duration in days	7.41 +/- 3	6 +/- 3.4	7.9 +/- 2.7		0.2
Mechanical ventilation (%)	74 (90%)	20 (9.1%)	54 (90.9%)	1.11 (0.207 – 5.965)	0.001
Average duration in days	11 +/- 14	5.75 +/- 5.56	12.94 +/- 15.7		0.006
prematurity	30 (36%)	11 (50%)	19 (31%)	2.182 (0.750 – 6.343)	2
IUGR (%)	47 (57%)	14 (63%)	33 (55%)	1.432 (0.523 – 3.918)	0.491
Prior antibiotictherapy	80 (97.5%)	20 (90%)	60 (100%)	0.250 (0.171 – 0.365)	5.00
3GC (%)	51 (62%)	12 (54%)	39 (65%)	0.646 (0.239 – 1.744)	0.748
Ampicillin(%)	27 (32%)	7 (31%)	20 (33%)	0.933 (0.328 – 2.655)	0.017
Gentamycin (%)	75 (91%)	17 (77%)	58 (96%)	0.117 (0.021 – 0.659)	7.755
Tienam (%)	65 (79%)	11 (50%)	54 (90%)	0.111 (0.034 – 0.364)	15.67
Amikacin (%)	66 (80%)	12 (54%)	54 (90%)	0.133 (0.041 – 0.438)	12.88
Patients whodied (%)	41 (50%)	12 (54%)	29 (48%)	0.780 (0.293 – 2.077)	0.248

Table 3: Distribution of bacterial clones according to risk factors

	Presence of the risk factor		Absence of the risk factor	
	Bacterial clones	**%**	**Bacterial clones**	**%**
	ST1/ST110/ST254/ST421/ST1158/ ST1382/ST1715	2,85%	ST478	2,85%
Mechanical	ST13/ST66/ST147/ST307/ST327	5,71%	ST1805	8,60%
ventilation	ST344	11,43%		
	ST478	8,60%		
	ST1805	20,00%		
	ST66/ST110/ST147/ST307	2,85%	ST1/ST66/ST147/ST254/ST307/ ST421/ST1158/ST1383/ST1715	2,85%
KTVO**	ST344	5,71%	ST13/ST327/ST344	5,71%
	ST1805	8,60%	ST478	11,43%
			ST1805	20,00%
	ST13/ST66/ST327/ST344/ST478/ST1383	2,85%	ST1/ST13/ST66/ST110/ST254/ ST327/ST421/ ST1158/ST1715	2,85%
IUGR*	ST147/ST307	5,71%	ST344/ST478	8,60%
	ST1805	17,14%	ST1805	11,43%
	ST1/ST110/ST254/ST344/ST421/ST1383	2,85%	ST1158/ST1715	2,85%
Prematurity	ST66/ST147/ST307/ST327/ST478	5,71%	ST13/ST478	5,71%
	ST1805	17,14%	ST344	8,60%
			ST1805	11,43%

**intra uterine growth retardation / **umbilical venous catheter*

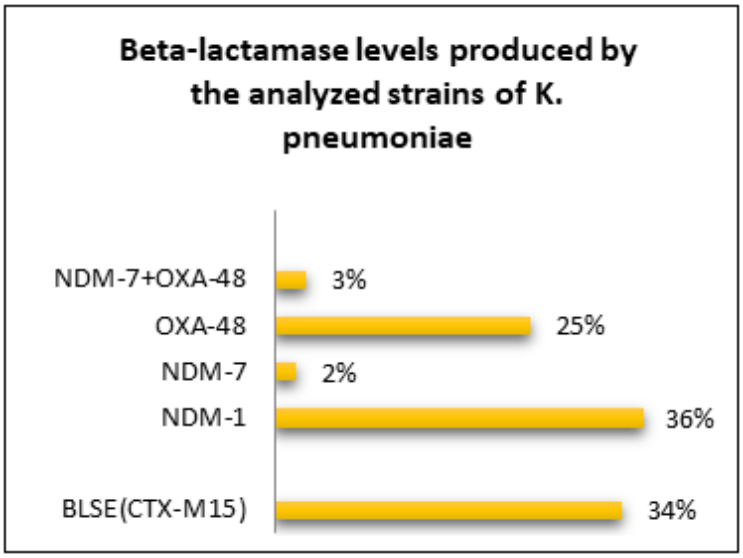

Figure 1: Beta-lactamase levels produced by the analyzed strains of K. pneumoniae

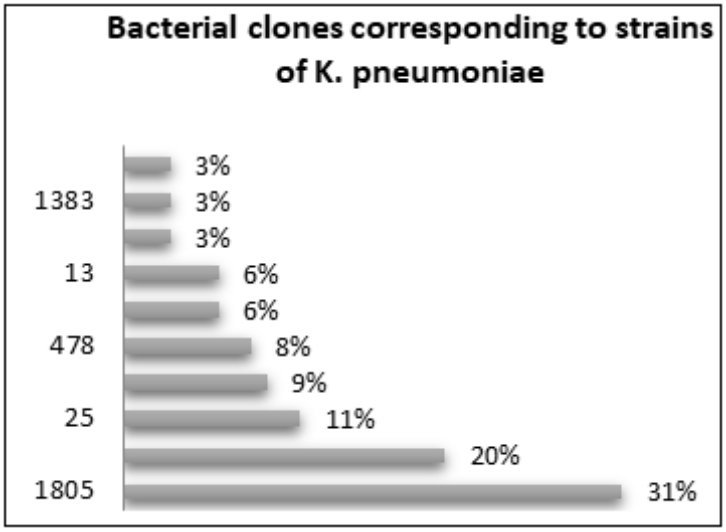

Figure 2: Bacterial clones corresponding to strains of K. pneumonia

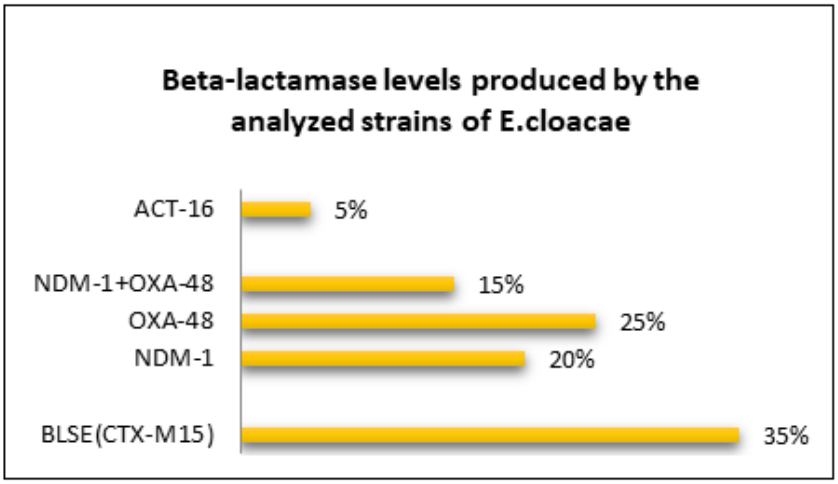

Figure 3: Beta-lactamase levels produced by the analyzed strains of E.cloacae

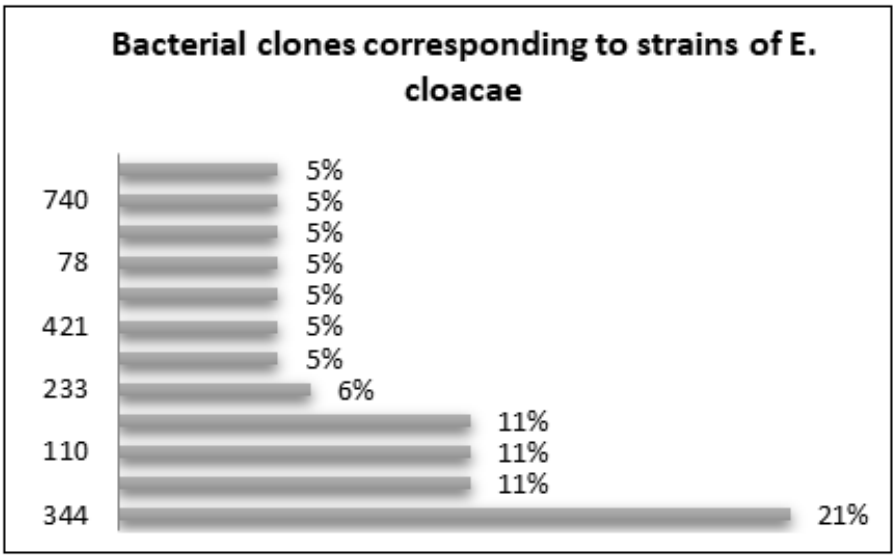

Figure 4: Bacterial clones corresponding to strains of E. cloacae

4. DISCUSSION

The incidence of neonatal infection in neonatology has been increasing for about ten years. Highly variable incidence rates are reported depending on the country and the type of unit. This variability also depends on the gestational age, the distribution of the children studied, the local environment and care practices [5]. In France, the prevalence of bacterial nosocomial infection in the neonatology department was estimated at 7.2% (5.4 per 1000 days of hospitalization) in the study by Sarlangue *et al*., [6]. An epidemiological study carried out in Casablanca by Hmamouchi *et al*., demonstrated a prevalence of 21.9% for an incidence density of 18.2 per 1000 days of hospitalization for neonatal nosocomial infections [7]. In the study conducted in Marrakech by Maoulainine *et al*., in 2012; the prevalence of late neonatal infections was 7.5% for an incidence density of 7.1 per 1000 days of hospitalization. As for our study, the prevalence of late bacterial infections in the neonatal intensive care unit; was 11.5% for an incidence density of 13.3 per 1000 days of hospitalization. Maoulainine *et al*., had reported in the study carried out in 2012 at the level of the neonatal intensive care unit of the CHU Mohamed VI in Marrakech; a male predominance with a sex ratio of 1.4 for nosocomial neonatal infections [8]. In Tunisia, a male predominance was noted in patients who presented with a nosocomial infection, in the study conducted by Merzougui et al, in the neonatology unit attached to the pediatric department of the CHU IBN El-Jazzar in Kairouan (sex- ratio at 1.55) [9]. A male predominance was also noted in our series (66.4% of cases of late MRB infections). This result is in good agreement with the literature data. In our study, IUGR was found in 55% of patients with late MRB infection.

In the study by Merzougui *et al*., conducted in the neonatology unit attached to the pediatric department of the CHU IBN El-Jazzar in Kairouan over a period of three years, BGN were the predominant germs (78.2%), followed by by Gram-positive cocci which represented 21.7% of germs. The most frequently found germ was K. Pneumoniae (41.0%), followed by E. coli (24.3%), then S. aureus (14.1%) [9]. In our study, the bacteria isolated from patients who had a late MRB infection were dominated by enterobacteriaceae, more particularly by K. pneumoniae (51.42%) and E. cloacae (37.15%). The other MRBs, namely K. oxytoca, P. oryzihabitans, AMRB and PAMR, were found at a lower frequency.

According to the literature, the length of hospitalization is a non-negligible risk factor; since 75% of nosocomial infections occur after the 6th day of hospitalization. In the study conducted by Merzougui *et al*., the length of hospitalization was identified as an independent risk factor (adjusted OR = 1.1). However, it is difficult to say whether this is the cause or the consequence of IN [9]. Regarding the average duration of hospitalization of late MRB infections in newborns, it was 17.47 days in our study. This is in good agreement with data from the literature. Newborns whose stay in intensive care is long are exposed to an increased risk of nosocomial infection. This association is moderately significant ($p<0.01$).

Mechanical ventilation is the major factor in the emergence of nosocomial pneumopathies. There is a close correlation between the incidence of pneumonia and the duration of intubation. The risk is major beyond 10 days of mechanical ventilation. In Egypt, a prospective incidence study showed that the use of mechanical ventilation multiplies by 5.4 the risk of developing a nosocomial infection in newborns [10]. This is the case of our study, since 90% of patients who presented a late MRB infection were on mechanical ventilation. This association is very significant ($p<0.001$). Among FDRs from INN to MRB in our

setting, the highest mortality rate was associated with the use of mechanical ventilation (89%).

A study conducted by Naas *et al*., at two hospitals located in Antananarivo Madagasar, 29 strains of E. cloacae and 15 ESBL-producing K. pneumoniae strains were analyzed using molecular biology techniques. The CTX-M15 gene was found in all strains [11]. In Turkey, a retrospective study was conducted in a university hospital in Istanbul, the strains of enterobacteriaceae isolated from these patients had shown a reduced sensitivity to carbapenems, in particular to imipenem, all the strains analyzed were producers of cabpenemase. Of these, 2 strains produced a KPC-2 type carbapenemase, 12 produced an NDM-1 type carbapenemase and 8 strains produced an OXA-48 type carbapenemase. The sequencing of the genome of these bacteria was carried out by MLST technique. The two strains of K. pneumoniae producing KPC-2 were associated with clone ST 307. The four strains of K. pneumoniae producing NDM-1 belonged to clones ST 15, ST 45, ST 278 and ST 1059, and the six strains of K. pneumoniae OXA-48 positive were associated with clone ST 101. The eight strains of E. NDM-1-producing cloacae were clonally indistinguishable [12].

An Italian study conducted by Gona *et al*., concerned 13 isolated strains of K. pneumoniae producing carbapenemases, in patients hospitalized in a neonatal intensive care unit at the University Hospital of Catania [13]. All the isolates analyzed co-produced two carbapenemases such as NDM-1 and OXA-48. Molecular analysis by MLST of these strains made it possible to identify mainly clone ST 101 and two new clones, namely ST 3666 and ST 3367.

Nayeem *et al*.,'s study included 17 carbapenem-resistant strains of K. pneumonia. All the isolated strains of K. pneumoniae were producers of NDM-type carbapenemases (including 13 NDM-1, 1 NDM-4 and 3 NDM-5). 76.5% of these strains co-produced a CTX-M-15 type carbapenemase, 41.2% co-produced an OXA-48, 41.2% co-produced a CMY-1 and 29.4% an SHV -1.

Among the 17 isolates of K. pneumoniae analyzed in this study, 6 bacterial clones were identified by MLST technique, including ST 15 (7 isolates), ST 16 (5 isolates), ST 11 (1 isolate), ST 657 (1 isolate), ST 873 (1 isolate) and ST 3344 (2 isolates). This study reported that NDM positive KP strains were associated with clones ST 15, ST 657 and ST 3344. While strains producing both NDM and OXA-48 belonged to clones ST 11, ST 16 and ST 873.

In our study, 66% of the analyzed strains of K. pneumoniae produced at least one carbapenemase. Of these, 38% produced an NDM-type carbapenemase (36% for NDM-1 and 2% for NDM-7), 25% produced an OXA-48-type carbapenemase and 3% co-produced 2 types of carbapenemases: NDM-7 and OXA-48. The ESBL production rate was 34% of cases. The sequencing of the genome of these strains by MLST technique, made it possible to identify 10 different bacterial clones, with predominance of clone ST 1805 (32%), followed by ST 307 (20%) and then ST 25 (11%). ST 1805 and ST 478 clones were associated with NDM production, ST 327 was associated with OXA-48 production, and ST 25 and ST 307 were associated with ESBL production.

Regarding the strains of E. cloacae analyzed in our study, 60% produced at least one carbapenemase. The distribution of carbapenemases found was as follows: overwhelmingly OXA-48 (25%), then NDM-1 (20%), followed by NDM-7+OXA-48 (15%). The ESBL type CTX-M15 production rate was 35%.

As for the analyzed strains of E. cloacae; the sequencing of their genomes also made it possible to identify 10 different bacterial clones, with the predominance of clone ST 344 (21%), followed by ST 1158, ST 110 and ST66 (11%). ST 344 was associated with OXA-48 production, ST 1158 was associated with both OXA-48 and NDM production, ST 110 was associated with ESBL and NDM production, and ST 66 was associated with the production of an NDM-1.

During the study period, the highest rates of resistance to antibiotics, in particular to carbapenems in the enterobacteriaceae analyzed, were observed in the following bacterial clones: ST 1805, ST 1158 and ST 307.

In our study, the bacterial clones identified, in the Enterobacteriaceae strains analyzed by the MLST technique, were predominated by the ST 1805 clone (28.7%), followed by the clones: ST 478 and ST 344 (11.6%).

A male predominance was noted in 71.4% of all the bacterial clones studied. Of all the bacterial clones analyzed, 88.5% were associated with VM, and 25.7% with KTVO. Prematurity was found in 62.8% of the bacterial clones, and 45.7% had presented an IUGR.

During the study period, the bacterial clones analyzed presented a heterogeneous distribution over time. The peak was recorded in August, September and November (20%). Of these, 65.7% were associated with a good evolution, against a death rate of 34.3%.

5. CONCLUSION

In conclusion, the emergence of multi-resistant germs in the neonatal environment constitutes today one of the most serious threats weighing on world health. This problem is of paramount importance,

warranting extensive research to adopt appropriate monitoring and prevention measures.

The current issue of multi-resistance in our context essentially affects enterobacteriaceae that are increasingly resistant to carbapenems.

Where does the interest of epigenetics come from, which seems to fit perfectly into the integrative and multidimensional direction that current microbiological work is taking. It is she who, by her perfection, constitutes the exception.

Based on the results of our study, and in the light of the bibliographical analysis, it seems essential to us to set up a strategy to fight against nosocomial infections and bacterial multiresistance to antibiotics in neonatal settings. Rationalizing the use of antibiotics, setting up a MRB monitoring system and a well-targeted prevention policy are measures whose urgent implementation is strongly recommended, in order to limit the emergence new strains of MRB in our institution.

BIBLIOGRAPHY

1. Clark, R., Powers, R., White, R., Bloom, B., Sanchez, P., & Benjamin, D. K. (2004). Nosocomial infection in the NICU: a medical complication or unavoidable problem?. *Journal of perinatology*, *24*(6), 382-388.
2. Chen, Y. C., Lin, C. F., Rehn, Y. J. F., Chen, J. C., Chen, P. Y., Chen, C. H., ... & Huang, F. L. (2017). Reduced nosocomial infection rate in a neonatal intensive care unit during a 4-year surveillance period. *Journal of the Chinese Medical Association*, *80*(7), 427-431.
3. Ghosh, D., Veeraraghavan, B., Elangovan, R., & Vivekanandan, P. (2020). Antibiotic Resistance and Epigenetics: More to It than Meets the Eye, *Antimicrob Agents Chemother*, 64(2).
4. Dupont, C., & Armant, D. R. (2009). Epigenetics: Definition, Mechanisms and Clinical Perspective. *Semin Reprod Med*, 27(5), 351-357.
5. Ahoyo, A. T., Baba-Moussa, L., Makoutode, M., Gbohoun, A., Bossou, R., & Dramane, K. (2006). Incidence of methicillin-resistant Staphylococcus aureus in the neonatology department of the departmental hospital center of Zou and Collines in Benin. *Arch Pediatr*, 13(11), 1391-1396.
6. Sarlangue, J., Hubert, P., Dageville, C., Boithias, C., & Gottot, S. (1998). Nosocomial infections in pediatrics. Epidemiological data, interest of networks. *Arch Pediatr*, (5), 191s-194s.
7. Hmamouchi, B., Chakkouri, K., Nejmi, S. E., & Chlilek, A. (2005). Epidemiology of nosocomial infection in pediatric intensive care units. *Annales Françaises d'Anesthesie et de Réanimation*, 24(6), 699-700.
8. Maoulainine, F. M., Elidrissi, N. S., Chkil, G., Abba, F., Soraa, N., Chabaa, L., ... & Aboussad, A. (2014). Epidemiology of nosocomial bacterial infection in a neonatal intensive care unit in Morocco. *Archives de Pediatrie: Organe Officiel de la Societe Francaise de Pediatrie*, *21*(9), 938-943.
9. Merzougui, L., Ben Helel, K., Hanachi, H., Metjaouel, H., & Brini, H. (2018). Risk factors for bacterial nosocomial infection in a neonatology center in the Tunisian Center. Case-control study: about 184 cases, *Journal de Pédiatrie et de Puériculture*, 31(1), 18-26.
10. Mohammed, D., & El Seifi, O. S. (2014). Bacterial nosocomial infections in neonatal intensive care unit, Zagazig University Hospital, Egypt. *Egyptian Pediatric Association Gazette*, *62*(3-4), 72-79.
11. Naas, T., Cuzon, G., Robinson, A. L., Andrianirina, Z., Imbert, P., Ratsima, E., ... & Raymond, J. (2016). Neonatal infections with multidrug-resistant ESBL-producing E. cloacae and K. pneumoniae in Neonatal Units of two different Hospitals in Antananarivo, Madagascar. *BMC Infectious Diseases*, *16*(1), 1-10.
12. Poirel, L., Yilmaz, M., Istanbullu, A., Arslan, F., Mert, A., Bernabeu, S., & Nordmann, P. (2014). Spread of NDM-1-producing Enterobacteriaceae in a neonatal intensive care unit in Istanbul, Turkey. *Antimicrobial agents and chemotherapy*, *58*(5), 2929-2933.
13. Gona, F., Bongiorno, D., Aprile, A., Corazza, E., Pasqua, B., Scuderi, M. G., ... & Mezzatesta, M. L. (2019). Emergence of two novel sequence types (3366 and 3367) NDM-1-and OXA-48-co-producing K. pneumoniae in Italy. *European Journal of Clinical Microbiology & Infectious Diseases*, *38*, 1687-1691.