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Profile of multidrug-resistant bacteria in neonatal nosocomial infections at the Brazzaville University Hospital, Congo

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Abstract:

Background: Antibiotic resistance represents a major threat in neonatology, particularly in sub-Saharan Africa where local data remain limited. The present study aims to establish the profile of multidrug-resistant bacteria isolated from cases of neonatal nosocomial infections at the Brazzaville University Hospital, Congo.
Methodology: This analytical cross-sectional study, conducted at the Brazzaville University Hospital on newborns with nosocomial infections between May and September 2023, characterized the bacterial organisms and their antimicrobial resistance profiles. Blood samples were collected from the neonates and cultured for bacterial isolation using conventional bacteriology methods. Antibiotic susceptibility test of each isolated bacterium was determined by the disc diffusion method and interpreted according to CA-SFM 2023 standards.
Results: Of 542 hospitalized patients during the period of study, 97 were neonates, 33 of whom had nosocomial infections, giving a prevalence of 34.0%. Bacteremia was the most common nosocomial infection with 78.8%, pneumonia (12.2%), urinary catheter infection (6.0%) and omphalitis (3.0%). Enterobacteriaceae, notably *Klebsiella pneumoniae* and *Escherichia coli*, dominated the infectious landscape, with marked multi-drug resistance (MDR) profile, nearly 80.0% of which is due to presumptive carbapenemase production. Gram-positive cocci and non-fermentative bacilli also present high resistance profiles. Factors significantly associated with nosocomial neonatal infection on multivariate analysis are low birthweight (<2500g) (OR=5.27, p=0.014) and requirement for oxygen (OR=8.12, p=0.003). But factors such as prematurity and the place of care of the newborns were not significantly associated with neonatal nosocomial infection on multivariate analysis (p>0.05).
Conclusion: These results highlight the vulnerability of newborns with low birth weight to nosocomial infection caused by MDR bacterial pathogen. There is need to strengthen antibiotic stewardship and integrate local data into hospital surveillance and infection prevention strategies.

Keywords: Nosocomial; Neonate; Infection; Multidrug resistance; Brazzaville-Congo

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Profil des bactéries multirésistantes dans les infections nosocomiales néonatales au CHU de Brazzaville, Congo

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Résumé:

Contexte: La résistance aux antibiotiques représente une menace majeure en néonatologie, particulièrement en Afrique subsaharienne où les données locales restent limitées. La présente étude vise à établir le profil des bactéries multirésistantes isolées chez des nouveau-nés atteints d'infections nosocomiales au CHU de Brazzaville, Congo.
Méthodologie: Cette étude transversale analytique, menée au CHU de Brazzaville auprès de nouveau-nés présentant des infections nosocomiales entre mai et septembre 2023, a caractérisé les bactéries isolées et leurs profils de résistance aux antimicrobiens. Des prélèvements sanguins ont été effectués chez les nouveau-nés et mis en culture pour l'isolement bactérien selon les méthodes bactériologiques conventionnelles. La sensibilité aux antibiotiques de chaque bactérie isolée a été déterminée par la méthode de diffusion sur disque et interprétée selon les normes CA-SFM 2023.
Résultats: Sur 542 patients hospitalisés pendant la période d'étude, 97 étaient des nouveau-nés, dont 33 présentaient une infection nosocomiale, soit une prévalence de 34,0%. La bactériémie était l'infection nosocomiale la plus fréquente (78,8%), suivie de la pneumonie (12,2%), de l'infection liée à une sonde urinaire (6,0%) et de l'omphalite (3,0%). Les entérobactéries, notamment *Klebsiella pneumoniae* et *Escherichia coli,* ont dominé le paysage infectieux, présentant un profil de multirésistance (MDR) marqué, dont près de 80.0% est dû à une production présumée de carbapénémases. Les cocci Gram positifs et les bacilles non fermentaires présentent également des profils de résistance élevés. Les facteurs significativement associés à l'infection néonatale nosocomiale en analyse multivariée sont un faible poids de naissance (<2500 g) (OR=5,27, p=0,014) et un besoin en oxygène (OR=8,12, p=0,003). En revanche, des facteurs tels que la prématurité et le lieu de prise en charge des nouveau-nés n'étaient pas significativement associés à l'infection néonatale nosocomiale en analyse multivariée (p>0,05).
Conclusion: Ces résultats soulignent la vulnérabilité des nouveau-nés de faible poids de naissance aux infections nosocomiales causées par des bactéries multirésistantes. Il est nécessaire de renforcer la gestion des antibiotiques et d'intégrer les données locales dans la surveillance hospitalière et les stratégies de prévention des infections.

Mots-clés: Infection nosocomiale; Nouveau-né; Infection; Multirésistance aux antibiotiques; Brazzaville-Congo

Introduction:

Antimicrobial resistance (AMR) is one of the most formidable challenges in global public health today. It is a complex phenomenon in which antimicrobial agents lose their effectiveness against microorganisms that have acquired defense mechanisms, often of genetic origin (1). Described as a "silent pandemic" by the World Health Organization (WHO), it was responsible for 1.27 million deaths worldwide in 2019, illustrating its devastating impact (2). In Europe, there are more than 33,000 deaths annually attributable to multidrug-resistant (MDR) bacteria (3), while in sub-Saharan Africa, this scourge is worsening in a context of limited resources, with an estimated mortality rate of 27.3 deaths per 100,000 inhabitants (4).

The hospital settings, particularly neonatal intensive care units, constitute high-risk environments for the emergence and spread of these resistant pathogens. Neonatal nosocomial infections (NNIs) refer to all infections acquired within a healthcare facility by newborns after admission. Their frequency depends on various factors such as early diagnosis, nature of invasive care, quality of microbiological control and patient immune profile. Although widely reported in both industrialized and developing countries, the incidence of NNIs varies significantly depending on the context. Estimated at 2.5% of hospitalized newborns in Europe (5), it reaches 15.8% in North Africa (6) and varies between 10% and 60% in sub-Saharan Africa (7).

In Congo, a study conducted at Brazzaville University Hospital reported a frequency of 1% of nosocomial neonatal bacterial infections among hospitalized newborns (8). Beyond their frequency, NNIs are associated with high morbidity and mortality, prolonged hospital stays, and significant treatment costs (9,10). The role of MDRs in these infections is worrying, with a distribution and resistance profile varying between units and regions. International data highlight this heterogeneity, in Iran, Rafati et al., (11) observed high resistance of Gram-negative bacilli to ceftazidime (72.7%), cefotaxime (54.5%), and ceftriaxone (60%); in Brazil, Limal et al., (12) reported that 14.9% of NNIs were attributable to MDRs.

At the Brazzaville University Hospital, the most frequently isolated bacterial pathogens in newborns are Gram-negative bacilli, including MDR *K. pneumoniae* (8), but local data remain incomplete regarding the resistance spectrum of the other pathogens involved. In this context, the present study aims to establish the profile of MDR bacteria isolated in cases of neonatal nosocomial infections at the Brazzaville University Hospital. This is to provide updated and contextualized data in order to strengthen therapeutic management, optimize antibiotic protocols, and contribute to the implementation of targeted surveillance and prevention strategies.

Materials and method:

Study design:

This analytical cross-sectional study was conducted at the neonatology and bacteriology-

immunology-virology departments of the University Hospital of Brazzaville, Congo, over a 4-month period (May–September 2023).

Ethical consideration:

The study respected the principles of confidentiality by anonymizing the data. Administrative authorizations were obtained from the Brazzaville University Hospital and the Research Ethics Committee (decision no. 054-40/MERSIT/DGRST/CERSSA/-23). Informed consent of the parents was systematically obtaimed. No medical risks were associated with participation.

Study population, participants and clinical data:

The study population included all neonates hospitalized during the period of study (n=97) from whom 33 neonates who have been hospitalized for more than 48 hours, with no documented infection at admission (CRP <20 mg/L and negative blood culture), and whose parents provided informed consent were included as study participants. Neonates with pre-analytical abnormalities or pre-existing neonatal infections were excluded.

Exhaustive blood sampling of each neonate was performed throughout the study period. A nosocomial infection was considered if the CRP exceeded 20 mg/L after 48 hours of hospitalization, associated with a positive blood culture (Fig 1).

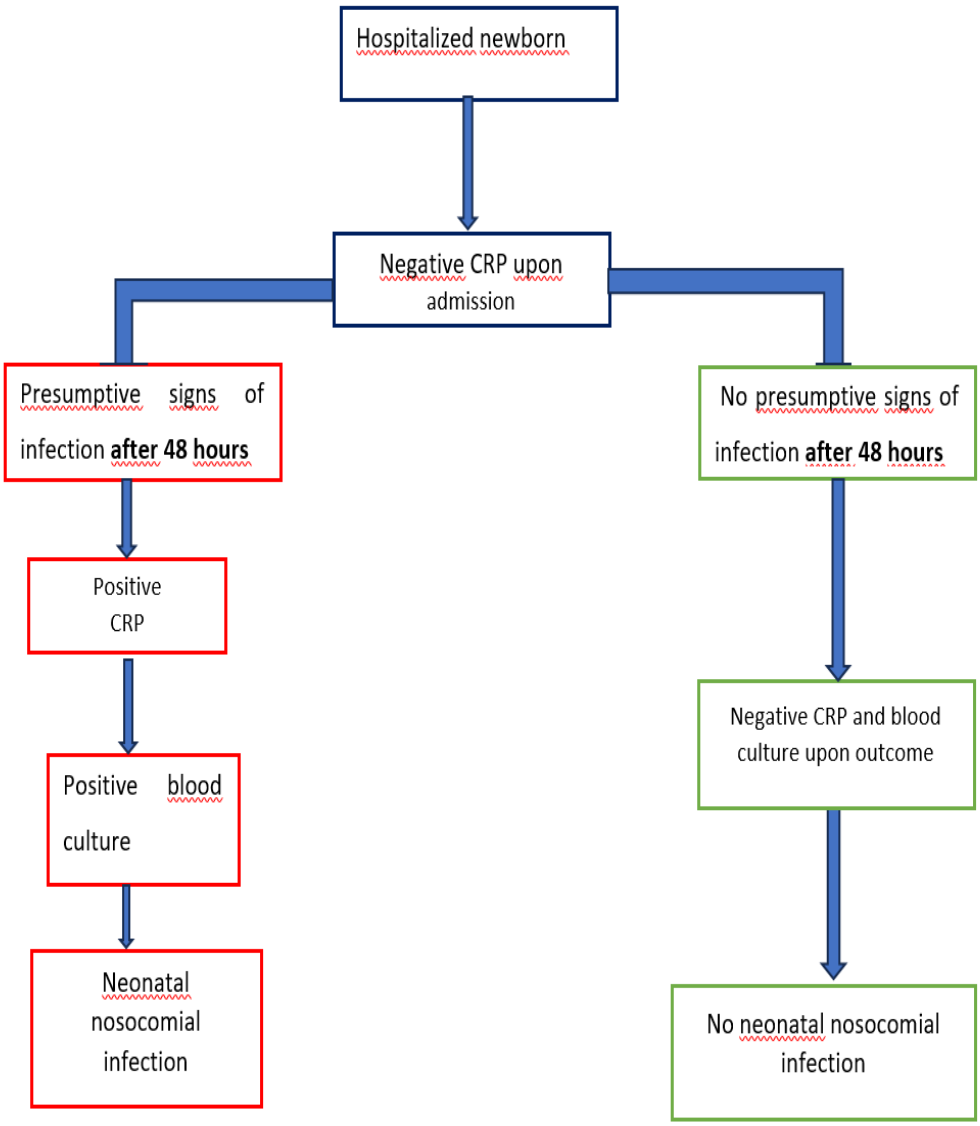


Fig 1: Diagnostic algorithm for neonatal nosocomial infections

Bacteriological analysis:

Blood culture:

For each neonate, 1-2 ml of whole blood was collected by peripheral venipuncture before antibiotic administration. Sampling was performed according to a strict protocol validated by the Hospital Infection Control Committee with the use of surgical mask, hand hygiene with an alcohol-based antiseptic solution, sterile gloves, disinfection of the puncture site (70% alcohol followed by an iodine-based product) and disinfection of the bottle septum.

The blood was inoculated into PF Plus bottles (bioMérieux) containing liquid medium that supports the growth of strict aerobic and facultative anaerobic bacteria. These bottles, equipped with a perforable rubber septum, were immediately transported to the laboratory. Blood culture bottles were incubated in a conventional incubator at 37°C for 5 days. Subcultures from the broth were performed on Mueller-Hinton agar at day 1, day 3, and day 5. Cultures remaining negative after 5 days were declared sterile; any culture positive at least once was retained for identification.

Bacterial identification and antibiotic Susceptibility:

Positive blood culture bottles were subcultured on solid culture media (Blood and MacConkey agar) and bacterial identification was performed using conventional methods (morphology, Gram staining, manual biochemical tests, Biomérieux® API galleries), in accordance with the laboratory's technical capabilities.

A standardized antibiotic susceptibity test (AST) was performed in Mueller-Hinton agar medium by the disc diffusion methd according to the CA-SFM 2023 recommendations. Selected antibiotic discs were tested in different classes; beta lactams, aminoglycosides, quinolones, nitrofurans, and trimethoprim-sulfamethoxazole. Inhibition zones were measured and interpreted to classify strains as sensitive, intermediate, or resistant in line with the CA-SFM 2023 guideline.

Phenotypic resistance detection:

Phenotypic detection of ESBLs was performed by double disc synergy testing with amoxicillin-clavulanic acid and 3rd generation cephalosporin discs. Carbapenemases and AmpC-type cephalosporinases were infered from the AST but not confirmed by specific or molecular tests due to lack of technical resources. Multi-drug-resistant in bacteria isolate was defined as resistance to ≥ 3 classes of antibiotics.

Statistical analysis:

Data were entered into CSPro 7.4 labtop computer and analyzed using SPSS version 24.0 and R software. Given the small sample size and the categorical nature of the variables studied, only Fisher's exact test was used to compare proportions between groups. The significance threshold was set at $p<0.05$.

Results:

Sociodemographic characteristics of newborns with nosocomial infection:

During the study period, 97 out of 542 hospitalized patients were included (54 females and 43 males). Among the 97 patients, 33 had nosocomial infection, giving a prevalence of 34.0%. Eighteen of the 54 (33.3%) females and 15 (34.9%) of the 43 males had nosocomial infection, but there was no significant difference in the prevalence of nosocomial infection with respect to sex (OR=0.9333, 95% CI=0.4011-2.1720), p=0.8728). Bacteremia was the most common nosocomial infection with 78.8% (n=26) followed by pneumonia (12.2%, n=4), catheter infection (6.0%, n=2) and omphalitis (3.0%, n=1) (Fig 2).

Factors associated wth neonatal nosocomial infections:

The prevalence of nosocomaial neonatal infections was significantly higher in neonates with birth weight <2500g (45.6%, n=26) than neonates with birth weigh >2.5g (21.9%, n=7) in both univariate (OR 3.00, p=0.029) and multivariate analyses (OR 5.27, p=0.014). The prevalence of nosocomaial neonatal infections was also significantly higher in neonates on oxygen therapy (48.3%, n=29) compared to those not on oxygen therapy (13.8%, n=4) in both univariate (OR 5.85, p=0.003) and multivariate analyses (OR 8.12, p=0.003. However, However, factors such as prematurity and the place of care of the newborns were not significantly associated with prevalence of neonatal nosocomial infection (Table 1).

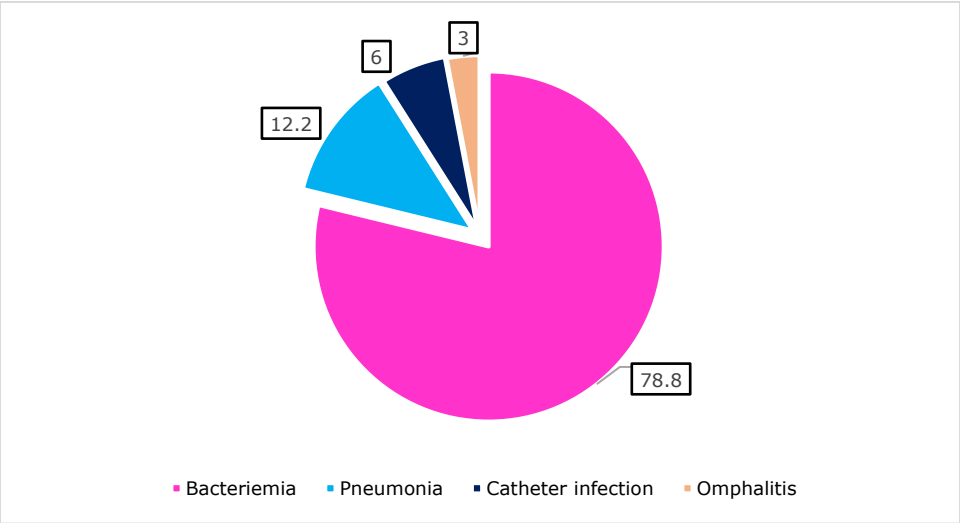


Fig 2: Types of neonatal nosocomial infections

Table 1: Univariate and multivariate analysis of risk factors for neonatal nosocomial infections

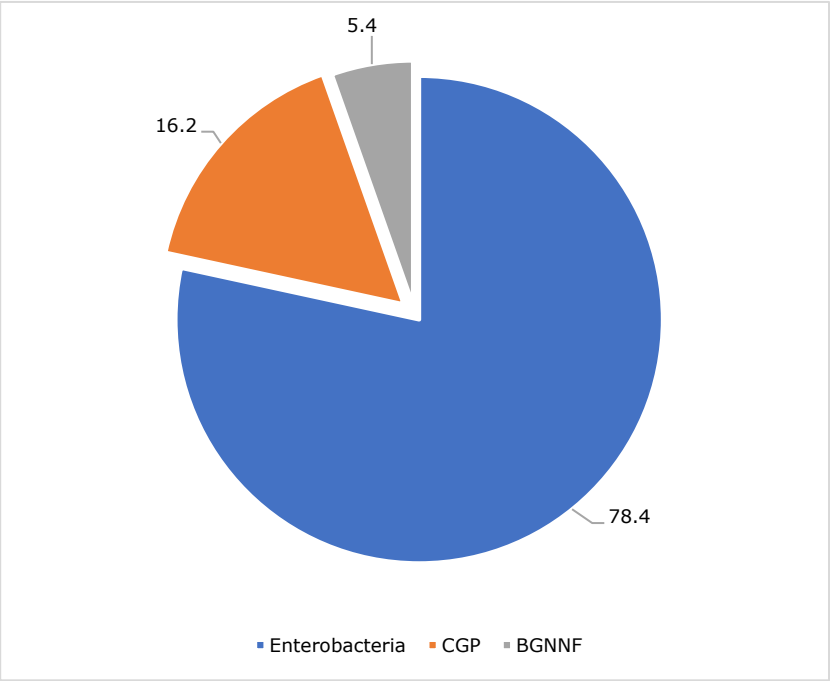
Risk factors	Nosocomial infection		OR (95% CI) *p*-value (univariable)	OR (95% CI) *p*-value (multivariable)
	Yes (n=33, 34.0%)	No (n=64, 66.0%)		
Birth weight (g)				
> 2500	7 (21.9)	25 (78.1)	3.00 (1.16-8.52) (*p*=0.029*)	5.27 (1.50-22.07) (*p*=0.014*)
< 2500	26 (45.6)	31 (54.4)		
Oxygen therapy				
No	4 (13.8)	25 (86.2)	5.85 (1.98-21.68) (*p*=0.003*)	8.12 (2.20-38.37) (*p*=0.003*
Yes	29 (48.3)	31 (51.7)		
Premature birth				
No	10 (27.0)	27 (73.0)	2.14 (0.88-5.48) (*p*=0.101)	0.59 (0.10-2.97) (*p*=0.526)
Yes	23 (44.2)	29 (55.8)		
Place of care				
Crib	10 (26.3)	28 (73.7)	2.48 (1.01-6.38) (*p*=0.05)	1.00 (0.19-5.34) (*p*=0.998)
Incubator	23 (46.9)	26 (53.1)		

* = Statistically significant at $p<0.05$

Frequency distribution of bacteria isolates of neonatal nosocomial infections:

A total of 37 bacteria were isolated from the 33 cases of neonatal nosocomial infections at the Brazzaville University Hospital and this is dominated by Enterobacteriaceae (78.4%, n= 29). Gram-positive cocci represent 16.2% (n=6) of isolates, while Non-Fermentative Gram-negative bacilli (NFGNB) are rare (5.4%, n=2) (Fig 3).

The most frequent members of the family Enterobacteriaceae, were *Klebsiella pneumoniae* (27%, n=10), *Escherichia coli* (16.3%, n=6), and *Enterobacter aerogenes* (10.8%, n=4) (Table 2). The distribution according to multi-drug resistant (MDR) bacteria strains is shown in Table 3, with 78.4 % (n=28) of the isolates being MDR.



NFGNB: Non-fermenting Gram-negative bacilli; GPC: Gram-positive cocci

Fig 3: Frequency distribution of bacterial isolates of neonatal nosocomial infections

Table 2: Distribution of bacterial isolates responsible for nosocomial infections

Bacterial isolates	**Frequency**	**Percentage (%)**
Enterobacteriaceae		
Klebsiella pneumoniae	10	27.0
Escherichia coli	6	16.3
Enterobacter aerogenes	4	10.8
Klebsiella spp	2	5.4
Serretia liquefasciens	2	5.4
Pantoea spp	2	5.4
Enterobacter cloacae	1	2.7
Raoutella terrigena	1	2.7
Raoutella ornithinolytica	1	2.7
NFGNB*		
Pseudomonas luteola	1	2.7
Pseudomonas aeruginosa	1	2.7
GPC**		
Staphylococcus spp	4	10.8
Staphylococcus aureus	1	2.7
Enterococcus spp	1	2.7
Total	**37**	**100.0**

NFGNB: Non-fermenting Gram-negative bacilli; GPC: Gram-positive cocci

Table 3: Distribution of multidrug-resistant bacteria (MDR) isolated from neonates with nosocomial infections at the Brazzaville University Hospital, Congo

Bacterial species	Total number of isolates	Number of MDR isolates	Percentage of MDR isolates
Klebsiella pneumoniae	10	10	100.0
Escherichia coli	6	6	100.0
Enterobacter aerogenes	4	4	100.0
Staphylococcus spp	4	3	75.0
Pantoea spp	2	2	100.0
Serratia liquefaciens	2	1	50.0
Raoultella terrigena	1	1	100.0
Staphylococcus aureus	1	1	100.0
Pseudomonas aeruginosa	1	1	100
Pseudomonas luteola	1	0	0
Enterococcus spp	1	0	0
Klebsiella spp	2	0	0
Raoutella terrigena	1	0	0
Raoutella ornithinolytica	1	0	0
Total	**37**	**29**	**78.4**

MDR: Multi-resistant bacteria (resistance to at least three families of antibiotics). Resistance involved the following main agents: ceftriaxone, ceftazidime, imipenem, tobramycin, ciprofloxacin, and pefloxacin.

Phenotype of bacterial resistance to β-lactam antibiotics:

Among the 23 multidrug-resistant Enterobacteriaceae identified, 18 (78.3%) expressed carbapenemase phenotype, indicating worrying resistance to carbapenems. High-level cephalosporinases were detected in 4 (17.4%) cases, while ESBLs was seen in only 1 (4.3%) strain. No wild-type phenotypes or single-penicillinase producers was observed.

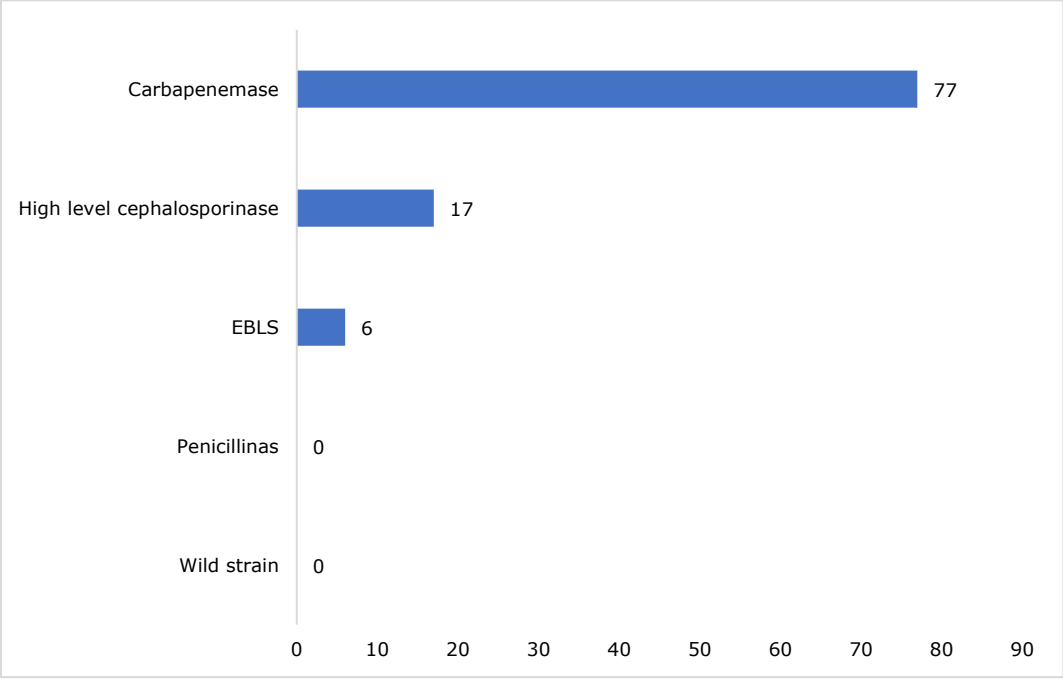


Fig 3: Phenotype of resistance of enterobacteria to beta-lactams

Discussion:

The frequency of neonatal nosocomial infection (NNI) in our study was 33.7%, similar to 33.0% reported by Lasme-Guillao et al., (13) in Abidjan. However, lower frequencies than ours have been reported in the United States and Europe, with rates ranging from 3.58% to 11.1% and 1.6% to 13.2%, respectively (14), and also in Africa, particularly in the Democratic Republic of Congo, with an incidence of 22.3%. These variations in frequency may be due to the criteria for defining NNI, the inclusion criteria, the sample size, and the study duration, which varied from one study to another, but also due to better hygiene conditions in Western countries.

We noted a slight female predominance in the study, with a sex ratio of 0.8. Chabni et al., (15) reported a sex ratio of 0.9, Bukasa et al., (14) also found a female predominance. The risk of developing NNI was higher in low birth weight newborns (p=0.029) which is identical to the findings of Auriti et al., (16) in Italy and Rangelova et al., (17) in Bulgaria. Indeed, low birth weight is characteristic of premature newborns, as these infants have immature immune system, making them more vulnerable to infection (18). The main site of nosocomial infections was blood with bacteremia in 78.8%, followed by pneumonia (12.2%). Our results are similar to those reported in the literature (18). Other localizations such as urinary tract infection, are very rarely diagnosed in our setting due to the low prevalence of urine cultures usually performed in neonatology.

Regarding factors associated with the occurrence of NNI, this study identified low birth weight, the use of oxygen therapy, and living in an incubator as factors associated with the occurrence of NNI. Newborns with a birth weight <2500g had a 3-fold increased risk of developing the infection in univariate analysis (OR= 3.00; p=0.029) and a 5-fold in multivariate analysis (OR=5.27; p=0.014). Those who were on oxygen therapy had a 6 times greater risk (OR =5.85; p=0.003) in univariate analysis and 8 times in multivariate analysis (OR=8.12; p= 0.003) of being infected by nosocomial bacterial pathogens. Newborns placed in an incubator also had a greater risk of developing a nosocomial infection in univariate analysis (OR=2.48; p=0.05) but not in multivariate analysis (OR= 1.00; p=0.998). The use of an incubator was also found to be a factor associated with the occurrence of infection in the study carried out in Madagascar on nosocomial bacteremia in neonatology (OR=6.78; 95% CI: 1.66-27.72; p=0.003) (19). Li Wang et al., (18) reported that low birth weight is a risk factor (OR=3.44, 95% CI: 2.31–5.11) among other factors such as use of mechanical ventilation (OR=3.16, 95% CI: 2.21–4.50) and gestational age of infection (OR=2.58).

The main bacterial pathogens isolated in our study were Gram-negative bacilli, particularly Enterobacteriaceae, dominated by *K. pneumoniae* (27.0%), followed by *E. coli* (17.0%) and *Enterobacter aerogenes* (10.0%). Gram-positive cocci were also isolated notably *Staphylococcus* spp in 10% of cases. Chemsi et al., (20) in Morocco, Ngakengni et al., (8) in Congo also reported *K. pneumoniae* as the main bacteria responsible for NNIs with respective frequencies of 37% and 50%. Among the isolated bacterial pathogens, none was found in its wild form; all the bacteria were therefore resistant. This resistance concerned the main antimicrobial molecules used in current practice in hospitals, such as ceftriaxone, ceftazidime, imipenem, tobramycin, ciprofloxacin, and pefloxacin but good activity was noted with gentamicin. These data thus call into question current standard therapeutic maanagement and shows the value of rapid blood culture with antibiotic sensitivity of isolated bacteria, with a view to better therapeutic management of patients with bacteremia.

The resistance to numerous antimicrobial molecules explains the prevalence of MDR bacteria in our study. These bacteria were MDR in 78.4 % of cases. All strains of *K. pneumoniae*, *E. coli* and *E. aerogenes* were MDR. The emergence and spread of antibiotic resistance represent a real threat to public health worldwide. Recent data in the literature report numerous cases of MDR or even totally-drug resistant (TDR) or pandrug resistant (PDR) bacteria to antibiotics, the number of which continues to increase both in industrialized and developing countries (20,21). The situation is alarming in resource-limited countries where infectious diseases, poverty, and malnutrition are endemic.

Regarding the phenotypes of resistance of Enterobacteriaceae to beta-lactams, they were dominated by the carbapenemase profile in 77.0% followed by the high-level cephalosporinase profile in 17.0% and the ESBL profile in 6.0% but we could not detect enzymes encoded by plasmid genes such as bla_{KPC}, bla_{NDM}, bla_{OXA-48}, which confer resistance to carbapenems (22,23) in our study due to lack of resources. This high prevalence of resistance profile is particularly worrying in neonatology, where therapeutic options are limited. However, the low prevalence of ESBL (4.3%) contrasts with the usual regional trends and could reflect a local evolution of the resistance profile or increased selective pressure linked to the intensive

use of carbapenems. This difference could be related to the small size of our sample but shows a worrying presence of MDR bacteria resistant to carbapenems. The presence of high-level cephalosporinases (17.4%) reinforces the therapeutic complexity, these enzymes being often inducible and not inhibited by clavulanic acid, which limits the effectiveness of classic combinations. In Congo Brazzaville, no study on the determination of carbapenemase genes has been carried out to date to highlight the different types.

With regard to these MDR strains, the therapeutic arsenal is extremely limited, particularly in pediatrics, requiring the administration of combinations of antibiotics which use in children is still poorly documented. Nevertheless, the present study allows us to propose a therapeutic protocol based on the bacterial ecology encountered in the neonatology department of CHU-B and the resistance phenotypes. However, it would be important to carry out the analysis of blood cultures and associate it with antibiotic susceptibility test results to confirm the detection of resistance. Thus, the use of therapeutic combinations such as ciprofloxacin and amikacin in cases of ESBL-producing bacteria and imipenem-relebactam/amikacin or meropenem-varobactam/amikacin could be used in case of MDR bacteria. However, this use should be better regulated, and blood cultures with antibiotic sensitivity tests are essential for diagnosis of MDR bacteria.

Conclusion:

This study highlights the high prevalence of MDR Enterobacteriaceae in neonatal nosocomial infections at the Brazzaville University Hospital, with a worrying predominance of carbapenemases as a resistance mechanism. Sociodemographic data confirm the increased vulnerability of premature and low birth weight neonates, highlighting the need for targeted prevention strategies.

The diversity of bacterial species isolated, including opportunistic and classic hospital pathogens, reflects a complex and dynamic ecology influenced by care practices, the hospital environment, and antibiotic pressure. The observed resistance profiles, particularly to β-lactams, fluoroquinolones, and macrolides, limit therapeutic options and call for a revision of empirical antibiotic protocols.

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Contributions of authors:

ENON conceived and designed the study, supervised data collection, and critically revised the manuscript; TM drafted the manuscript, performed data analysis, and coordinated the revision process; LM conducted bacteriological analyses and contributed to laboratory data interpretation; NN assisted in clinical data collection in neonatology and contributed to manuscript revision; OEN participated in data acquisition and contributed to statistical analysis; and GE provided clinical expertise in neonatology, validated results, and contributed to the discussion section. All authors approved the manuscript submit for publication.

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Conflict of interest:

No confloict of interest is declared.

Data availability:

The dataset of this study is available from the corresponding author on reasonable request.

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