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Molecular detection of antimicrobial resistance genes in multidrug-resistant Gram-negative bacteria isolated from clinical samples in two hospitals in Niger

*[1]Abdoulaye, O., [2]Abdoulaye, I., [3]Alassane Halawen, M., [4]Ibrahim Mamadou, A. K., [1,5]Maman Sani Falissou, S., [5,6]Adamou Amatagas, S., [1]Boureima, H., [2]Boubacar Issaka, B., [2]Ide, H., [7]Yacouba, A., [1,5]Sidi Maman Bacha, B., [3]Chaibou, S., [2]Hamadou, I., [1]Harouna Amadou, M. L., [2]Ousmane, S., [5]Doutchi, M., and [7]Mamadou, S.

[1]Faculté des Sciences de la Santé, Université de Dan Dicko Dankoulodo de Maradi, BP 465, Niger
[2]Centre de Recherche Médicale et Sanitaire, Niamey, Niger
[3]Hôpital Général de Référence de Niamey, Niger
[4]Centre Hospitalier Régional de Dosso, Niger
[5]Hôpital National de Zinder, Niger
[6]Faculté des Sciences de la Santé, Université André Salifou de Zinder, Niger
[7]Faculté des Sciences de la Santé, Université Abdou Moumouni, Niamey, Niger
*Correspondence to: ousmaneabdoulaye2010@yahoo.com; Cel: (+227) 96354580

Abstract:

Background: According to the World Health Organization (WHO), bacterial resistance to antibiotics is a global public health challenge, which is also developing in Niger. The aim of this study was to determine the prevalence of antibiotic resistance genes in Gram-negative bacilli isolated from clinical samples in the biological laboratories of two selected health facilities in Niger.
Methodology: Clinical bacterial isolates were randomly collected from two biological laboratories of Zinder National Hospital and Niamey General Reference Hospital. These were multi-resistant Gram-negative bacteria that have been routinely isolated from pathological samples of patients. Molecular detection of resistance genes was carried out by polymerase chain reaction (PCR) amplification using specific primers. These include plasmid-mediated AmpC beta lactamase genes (*bla*$_{CITM}$, *bla*$_{DHAM}$, *bla*$_{FOXM}$), 'Cefotaxime-Munich' type beta lactamase genes (*bla*$_{CTX-M-1}$, *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-9}$), KPC-type beta lactamase gene (*bla*$_{KPC}$), Oxa-type beta lactamase gene (*bla*$_{OXA-48}$), SHV-type beta lactamase gene (*bla*$_{SHV}$), TEM-type beta lactamase gene (*bla*$_{TEM}$), quinolone resistance genes (*qnrA*, *qnrB*, *qnrS*), and sulfonamide resistance genes (*sul1*, *sul2*, *sul3*).
Results: A total of 24 strains of multidrug-resistant Gram-negative bacteria isolated from different clinical samples were analysed. The distribution of the resistance genes detected is as follows; AmpC *bla*$_{CITM}$ (n=6; 25.0%), AmpC *bla*$_{DHAM}$ (n=4; 17.0%), AmpC *bla*$_{FOXM}$ (n=0), *bla*$_{CTX-M-1}$ (n=11; 46.0%), *bla*$_{CTX-M-2}$ (n=0), *bla*$_{CTX-M-9}$ (n=0), *bla*$_{KPC}$ (n=0), *bla*$_{OXA-48}$ (n=2; 8..0%), *bla*$_{SHV}$ (n=5; 21.0%), *bla*$_{TEM}$ (n=0), *qnrA* (n=0), *qnrB* (n=5; 21.0%), *qnrS* (n=17; 71.0%), *sul1* (n=22; 92.0%), *sul2* (n=12; 50.0%), and *sul3* (n=0). All isolates tested had at least two resistance genes.
Conclusion: The results of this study provide a better understanding of the resistance situation of clinical isolates in Niger. Therefore, it is more than necessary to intensify the detection on a larger number of samples and on a national scale. This will make it possible to assess the true extent of the phenomenon and consequently guide control strategies through a national multisectoral plan.

Keywords: Gram-negative bacilli, Resistance genes, PCR, Hospitals, Niger.

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Détection moléculaire de gènes de résistance aux antimicrobiens chez des bactéries Gram-négatives multirésistantes isolées à partir d'échantillons cliniques dans deux hôpitaux du Niger

*[1]Abdoulaye, O., [2]Abdoulaye, I., [3]Alassane Halawen, M., [4]Ibrahim Mamadou, A. K., [1,5]Maman Sani Falissou, S., [5,6]Adamou Amatagas, S., [1]Boureima, H., [2]Boubacar Issaka, B., [2]Ide, H., [7]Yacouba, A., [1,5]Sidi Maman Bacha, B., [3]Chaibou, S., [2]Hamadou, I., [1]Harouna Amadou, M. L., [2]Ousmane, S., [5]Doutchi, M., et [7]Mamadou, S.

[1]Faculté des Sciences de la Santé, Université de Dan Dicko Dankoulodo de Maradi, BP 465, Niger
[2]Centre de Recherche Médicale et Sanitaire, Niamey, Niger
[3]Hôpital Général de Référence de Niamey, Niger
[4]Centre Hospitalier Régional de Dosso, Niger
[5]Hôpital National de Zinder, Niger
[6]Faculté des Sciences de la Santé, Université André Salifou de Zinder, Niger
[7]Faculté des Sciences de la Santé, Université Abdou Moumouni, Niamey, Niger
* Correspondance à: ousmaneabdoulaye2010@yahoo.com; Cel: (+227) 96354580

Résumé:

Contexte: Selon l'Organisation Mondiale de la Santé (OMS), la résistance bactérienne aux antibiotiques constitue un défi mondial de santé publique, qui se développe également au Niger. Le but de cette étude était de déterminer la prévalence des gènes de résistance aux antibiotiques chez les bacilles Gram négatif isolés à partir d'échantillons cliniques dans les laboratoires de biologie de deux formations sanitaires sélectionnées au Niger.
Méthodologie: Des isolats bactériens cliniques ont été collectés de manière aléatoire dans deux laboratoires de biologie de l'Hôpital National de Zinder et de l'Hôpital Général de Référence de Niamey. Il s'agissait de bactéries Gram-négatives multirésistantes qui ont été systématiquement isolées à partir d'échantillons pathologiques de patients. La détection moléculaire des gènes de résistance a été réalisée par amplification par réaction en chaîne par polymérase (PCR) à l'aide d'amorces spécifiques. Il s'agit notamment des gènes de bêta-lactamase AmpC à médiation plasmidique (*bla*$_{CITM}$, *bla*$_{DHAM}$, *bla*$_{FOXM}$), des gènes de bêta-lactamase de type «Céfotaxime-Munich» (*bla*$_{CTX-M-1}$, *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-9}$), du gène de bêta-lactamase de type KPC (*bla*$_{KPC}$), du gène de bêta-lactamase de type Oxa (*bla*$_{OXA-48}$), le gène bêta-lactamase de type SHV (*bla*$_{SHV}$), le gène bêta-lactamase de type TEM (*bla*$_{TEM}$), les gènes de résistance aux quinolones (*qnrA, qnrB, qnrS*) et les gènes de résistance aux sulfamides (*sul1, sul2, sul3*).
Résultats: Au total, 24 souches de bactéries Gram-négatives multirésistantes isolées de différents échantillons cliniques ont été analysées. La répartition des gènes de résistance détectés est la suivante; AmpC *bla*$_{CITM}$ (n=6; 25,0%), AmpC *bla*$_{DHAM}$ (n=4; 17,0%), AmpC *bla*$_{FOXM}$ (n=0), *bla*$_{CTX-M-1}$ (n=11; 46,0%), *bla*$_{CTX-M-2}$ (n=0), *bla*$_{CTX-M-9}$ (n=0), *bla*$_{KPC}$ (n=0), *bla*$_{OXA-48}$ (n=2; 8,0%), *bla*$_{SHV}$ (n=5; 21,0%), *bla*$_{TEM}$ (n=0), *qnrA* (n=0), *qnrB* (n=5; 21,0%), *qnrS* (n=17; 71,0%), *sul1* (n=22; 92,0%), *sul2* (n=12; 50,0%) et *sul3* (n=0). Tous les isolats testés possédaient au moins deux gènes de résistance.
Conclusion: Les résultats de cette étude permettent de mieux comprendre la situation de résistance des isolats cliniques au Niger. Il est donc plus que nécessaire d'intensifier la détection sur un plus grand nombre d'échantillons et à l'échelle nationale. Cela permettra d'évaluer l'ampleur réelle du phénomène et par conséquent d'orienter les stratégies de lutte à travers un plan national multisectoriel.

Mots clés: Bacilles Gram négatif, Gènes de résistance, PCR, Hôpitaux, Niger.

Introduction:

Antimicrobial resistance (AMR) is a global and sub-regional problem. Today, the evolution and spread of bacterial resistance is evident from the results observed in the field (1,2). As in other West African countries, AMR is growing exponentially in Niger, and threatens patient care, economic growth, public health, agriculture and, most importantly, economic security (3).

One of the main limitations in assessing the extent of the phenomenon is the lack of data from field studies. Indeed, it is from these data that strategies to combat bacterial resistance to antibiotics should be derived, in particular data on the epidemiology of multi-resistant (MDR) bacterial strains. In this context, we set out to determine the prevalence of antibiotic resistance genes in pathogenic and MDR Gram-negative bacteria commonly isolated in healthcare facilities in Niger.

Materials and method:

Study sites:

The study was conducted at the Niamey General Reference Hospital, the Zinder National Hospital and the Centre de Recherche Sanitaire et Médicale (CERMES), Niamey.

Study type and period:

This was a laboratory-based study to detect the genes mediating resistance in MDR Gram-negative bacteria isolated from clinical samples of infected patients in two hospitals in Niger from June 2021 to December 2022. Characterization of antibiotic resistance genes was performed at CERMES, Niamey, Niger in October 2023.

Ethical approval:

The study was approved by the ethics committees of both hospitals for the use and analysis of data from medical laboratories. Anonymity and confidentiality of the data were guaranteed.

Bacterial isolates collection:

Clinical bacterial isolates were collected randomly from two biological laboratories in National Hospital in Zinder and General Reference Hospital in Niamey. The isolates were MDR Gram-negative bacteria isolated from pathological samples of infected patients for diagnostic purposes.

DNA extraction:

Genomic DNA was extracted from the various isolates using the "heat shock" method. Four to five young colonies obtained by subculture were resuspended in 200µl sterile

distilled water and placed at -20°C for at least 30 minutes. The suspension was then heated to boiling (100°C) for 10 minutes and centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant obtained was used as DNA template.

PCR amplification:

Simplex PCR assay was used. A negative control consisting of extraction water and a positive control were used for all gene amplification reactions. The 22.5µl reaction mix consisted of 12.5µl Quanta® Master, 1.5µl forward (sense) and reverse (antisense) primers (Table 1) and 7µl water. A volume of 2.5µl of DNA extract was added to the reaction mix to give a final volume of 25µl in each of the appropriate wells and a thermal cycle in a thermocycler of 95°C for 5 minutes (initial denaturation) followed by 35 cycles of 94°C for 30 sec (denaturation), a variable hybridization temperature depending on the primer for 30 sec and 72°C for 60 seconds for primer extension (Table 1).

Detection of the PCR reaction:

The PCR products were migrated on a 2% agarose gel prepared in TAE (Tris-EDTA) buffer and visualized using a Trans illuminator to show the expected sizes using a molecular weight scale (DNA molecular weight 100µg, Promega).

Table 1: Detail of the primers used in the study

Gene	Primer sequence	Hybridization temperature	Amplicon size	Reference
bla$_{TEM}$	F - ATA-AAA-TTC-TTG-AAG-ACG-AAA R - GAC-AGT-TAC-CAA-TGC-TTA-ATC-A	40.8°C	861 pb	AB282997
bla$_{SHV}$	F - CGC-CGG-GTT-ATT-CTT-ATT-TGT-CGC R - TCT-TTC-CGA-TGC-CGC-CGC-CAG-TCA	54.85°C	927 pb	X98098
bla$_{KPC}$	F - CGT-TCT-TGT-CTC-TCA-TGG-CC R - CCT-CGC-TGT-GCT-TGT-CAT-CC	63°C	798 pb	Poirel et al., (4)
bla$_{OXA-48}$	F - TTG-GTG-GCA-TCG-ATT-ATC-GG R - GAG-CAC-TTC-TTT-TGT-GAT-GGC	53.65°C	281 pb	Poirel et al., (4)
bla$_{CTX-M-1}$	F - GGT-TAA-AAA-ATC-ACT-GCG-TC R - TTG-GTG-ACG-ATT-TTA-GCC-GC	58°C	944 pb	X92506
bla$_{CTX-M-2}$	F - ATG-ATG-ACT-CAG-AGC-ATT-CG R - TGG-GTT-ACG-ATT-TTC-GCC-GC	60°C	351 pb	X92507
bla$_{CTX-M-9}$	F - ATG-GTG-ACA-AAG-AGA-GTG-CA R - CCC-TTC-GGC-GAT-GAT-TCT-C	59°C	876 pb	AF174129
AmpC *bla*$_{FOXM}$	F - AAC-ATG-GGG-TAT-CAG-GGA-GAT-G R - CAA-AGC-GCG-TAA-CCG-GAT-TGG	50.45°C	190 pb	X77455
AmpC *bla*$_{CITM}$	F - TGG-CCA-GAA-CTG-ACA-GGC-AAA R - TTT-CTC-CTG-AAC-GTG-GCT-GGC	50.2°C	465 pb	X78117
AmpC *bla*$_{DHAM}$	F - AAC-TTT-CAC-AGG-TGT-GCT-GGG-T R - CCG-TAC-GCA-TAC-TGG-CTT-TGC	50.45°C	405 pb	Y16410 AF189721
qnrA	F - TTC-TCA-CGC-CAG-GAT-TTG-AG R - TGC-CAG-GCA-CAG-ATC-TTG-AC	61°C	516 pb	AY070235
qnrB	F - TGG-CGA-AAA-AAA-TTR-ACA-GAA R - GAG-CAA-CGA-YGC-CTG-GTA-G	52°C	526 pb	DQ351241
qnrS	F - GAC-GTG-CTA-ACT-TGC-GTG-AT R - AAC-ACC-TCG-ACT-TAA-GTC-TGA	52.65°C	417 pb	DQ485529
sul1	F - CGG-CGT-GGG-CTA-CCT-GAA-CG R - GCC-GAT-CGC-GTG-AAG-TTC-CG	67°C	433 pb	AY655484.1
sul2	F - GCG-CTC-CAA-GGC-AGA-TGG-CAT-T R - GCG-TTT-GAT-ACC-GGC-ACC-CGT	53.3°C	293 pb	AY360321.1
sul3	F - GGA-AGA-AAT-CAA-AAG-ACT-CAA R - CCT-AAA-AAG-AAG-CCC-ATA-CC	49.7°C	569 pb	AJ459418.2

Data analysis:

Data were collected using a standard biological information form. Analysis was performed using Excel 2013 software and results were presented as tables and figures.

Results:

Of the 24 MDR Gram-negative bacterial isolates collected from pathological specimens, *Escherichia coli* was the most frequent accounting for 58.3% (n=14). All strains were extended-spectrum beta-lactamase (ESBL) phenotype except one, which was MDR *Pseudomonas aeruginosa* (MDRPA) (Table 2).

Prevalence of resistance genes:

The frequency of detection of resistance genes in the bacterial isolates is shown in Fig 1 with; (i) Plasmid-mediated AmpC beta lactamases: AmpC bla_{CITM} (n=6, 25.0%), AmpC bla_{DHAM} (n=4, 17.0%), and AmpC bla_{FOXM} (n=0); (ii) 'Cefotaxime-Munich' type beta lactamases: $bla_{CTX\text{-}M\text{-}1}$ (n=11, 46.0%), $bla_{CTX\text{-}M\text{-}2}$ (n=0), and $bla_{CTX\text{-}M\text{-}9}$ (n=0); (iii) KPC-type beta lactamases: bla_{KPC} (n=0); (iv) Oxa-type beta lactamases: $bla_{OXA\text{-}48}$ (n=2, 8.0%); (v) SHV-type beta lactamases: bla_{SHV} (n=5, 21.0%); (vi) TEM-type beta-lactamases: bla_{TEM} (n=0); (vii) Quinolone resistance genes: *qnrA* (n=0), *qnrB* (n=5, 21.0%), and *qnrS* (n=17, 71.0%); and (viii) Sulfonamide resistance genes: *sul1* (n=22, 92.0%), *sul2* (n=12, 50.0%), and *sul3* (n=0).

Fig 2 shows the gel electrophoresis pictures of the 16 amplified genes across the 24 bacterial isolates.

Table 2: Source and antibiotic phenotype profiles of bacterial isolates in the study

Serial No	Isolate No	Sample source	Isolate genus/species	Antibiotic phenotype
1	2245HGR	Urine	*Escherichia coli*	ESBL
2	2543HGR	Urine	*Klebsiella oxytoca*	ESBL
3	2543HGR	Urine	*Escherichia coli*	ESBL
4	2548HGR	Urine	*Escherichia coli*	ESBL
5	2559HGR	Urine	*Escherichia coli*	ESBL
6	1800HGR	Urine	*Escherichia coli*	ESBL
7	2258HGR	Urine	*Pseudomonas aeruginosa*	ESBL
8	1644HNZ	Urine	*Escherichia coli*	ESBL
9	1595HNZ	Urine	*Escherichia coli*	ESBL
10	1597HNZ	Urine	*Serratia odorifera*	ESBL
11	1567HNZ	Urine	*Escherichia coli*	ESBL
12	1215HNZ	Urine	*Pseudomonas aeruginosa*	ESBL
13	1599HNZ	Urine	*Escherichia coli*	ESBL
14	1791HNZ	Urine	*Klebsiella pneumoniae*	ESBL
15	1539HNZ	Urine	*Escherichia coli*	ESBL
16	1878HNZ	Urine	*Klebsiella oxytoca*	ESBL
17	1662HNZ	Urine	*Serratia odorifera*	ESBL
18	1601HNZ	Urine	*Pseudomonas aeruginosa*	MDRPA
19	1657HNZ	Urine	*Klebsiella oxytoca*	ESBL
20	118HGR	Pus	*Escherichia coli*	ESBL
21	148HGR	Pus	*Escherichia coli*	ESBL
22	175HGR	Urine	*Escherichia coli*	ESBL
23	166HGR	Pus	*Escherichia coli*	ESBL
24	1791HGR	Urine	*Klebsiella pneumoniae*	ESBL

ESBL: Extended spectrum b-lactamase; MDRPA: Multi-drug resistant *Pseudomonas aeruginosa*

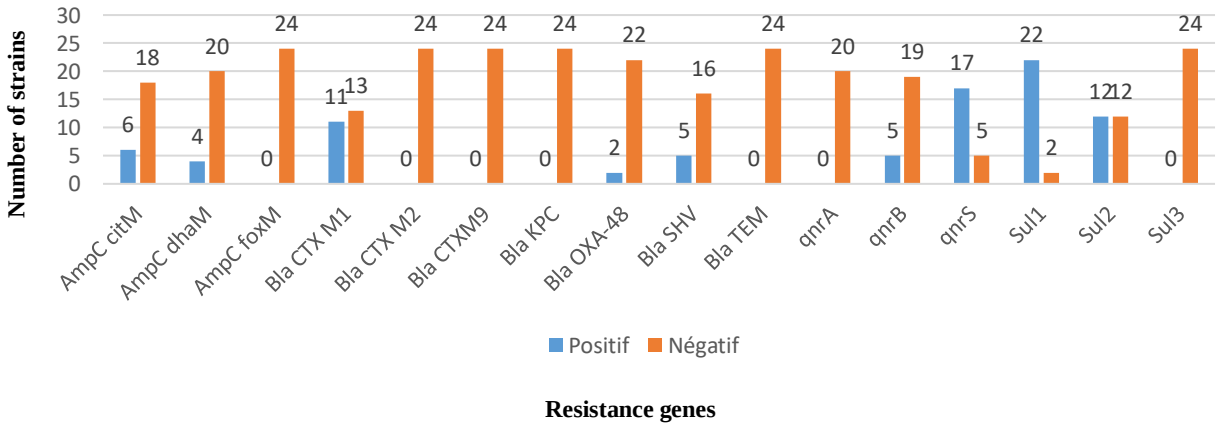
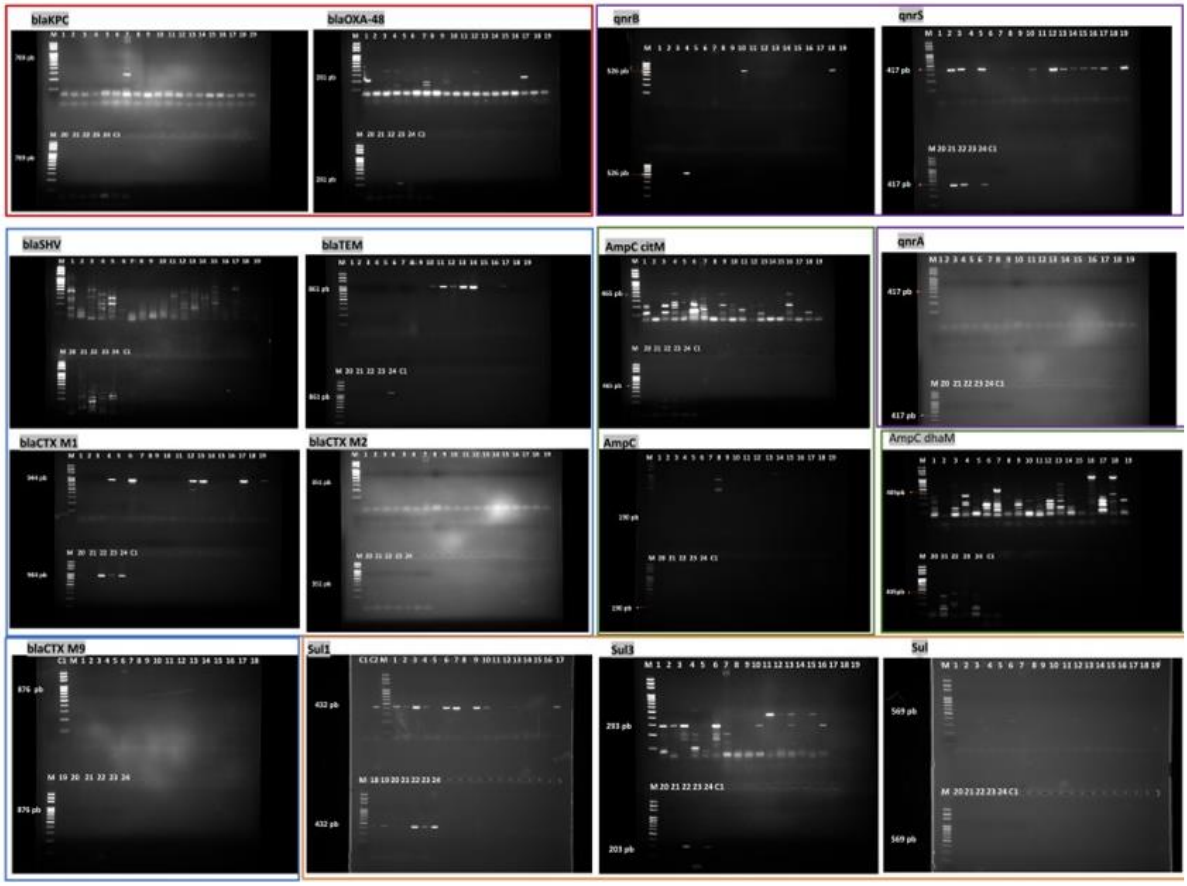


Fig 1: Prevalence distribution of the 16 resistance genes in the bacterial isolates



C1: Negative control; C2: Positive control; M: Size marker; Bacterial isolates: 1, 2, 3, 4, 5, 6; 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24.

Fig 2: Gel electrophoresis pictures of the 16 amplified genes across the bacterial isolates

Discussion:

The threat to public health posed by AMR remains a major and growing challenge, especially in Gram-negative bacteria (5). The aim of our study was to identify the genetic determinants of resistance to different families of antibiotics in pathogenic and MRD Gram-negative bacteria in the biology laboratory of the National Hospital of Zinder and the General Reference Hospital of Niamey in Niger. Of the total of 24 MDR GNB isolates from different clinical samples, all (100%) the isolates carried at least two resistance genes, confirming them to be multidrug resistant.

The AmpC bla_{CITM} gene represented 25.0% and AmpC bla_{DHAM} 17.0% of our isolates. A similar study conducted in Nigeria reported that 4.5% (6/134) of the strains studied carried plasmid-mediated AmpC variants; bla_{ACT-1}, bla_{DHA-1} and bla_{CMY-2} in 1, 4 and 1 isolates respectively (6). It should be noted that AmpC (ampicillin class C beta-lactamase) genes, in particular *MOXM, CITM, DHAM, EBCM, FOXM* and *ACCM*, are responsible for the development of broad-spectrum resistance to most β-lactams except cefepime and carbapenems (7).

CTX-M-type β-lactamases are among the most common and widespread ESBLs, mainly found in Enterobacteriaceae (8). Although discovered later than TEM- and SHV-type ESBLs, it is now clear that CTX-M-type β-lactamases play an important role as emerging resistance determinants in Enterobacteriaceae (8,9). In our study, the gene encoding CTX-M-1-type β-lactamases ($bla_{CTX-M-1}$) was found in almost half of the isolates (46.0%, n=11). The study conducted in Nigeria by Ogbolu et al., (6) reported comparably lower rate of 18.7% (25 out of 134 isolates). In addition, the study by Bonnet (10) reported a worldwide distribution of these enzymes. In a study conducted in central Iran, Abbassi et al., (11) also reported that carbapenem-resistant *Klebsiella pneumoniae* (CRKP) strains contained $bla_{CTX-M-1}$ (100.0%) and $bla_{CTX-M-15}$ (96%) genes. Similar results to ours were also reported by Omneya and Mona (9) in Egypt, where the ESBL resistance genes $bla_{CTX-M-15}$ and $bla_{CTX-M-14}$ were detected in 60.0% and 24.0% isolates, respectively.

In our study, the bla_{SHV} was seen in 5 out of the 24 (21.0%) isolates tested, mainly in *E. coli*, *S. odorifera*, *Pseudomonas* spp and *K. pneumoniae*. A relatively low prevalence

(6.7%, 30/128) was reported by Socohou et al., in Benin (12). However, Codjoe et al., (13) reported that all carbapenemase-producing strains (100.0%) carried the *bla*$_{TEM}$ and *bla*$_{SHV}$ ESBL genes in their study conducted in Ghana. The *bla*$_{OXA-48}$ gene was found in our study with a frequency of 8.0% (2/24). These results are comparable to those reported by Elbadawi et al., (14) in Sudan and Dikoumba et al., (15) in Gabon with frequencies of 2.4% (5/121) and 40%, respectively. In 2014, Dortet et al., (6) reported that OXA-48-like enzymes accounted for 86.0% of a panel of more than 1,000 French carbapenemase-producing Enterobacteriaceae (EPC) strains, well ahead of the New Delhi metallo-beta-lactamases (NDM) of 8%.

In South Africa, a review of the literature on antibiotic resistance reported that the NDM-1 and OXA-48 genotypes were the most common in Gram-negative bacteria involved in clinical infections (7). This *bla*$_{OXA-48}$ gene, which belongs to class D of the Ambler class has hydrolytic activity towards penicillins and carbapenems, including imipenem, ertapenem and meropenem, but little or no activity towards third-generation cephalosporin (8). Given this diversity of carbapenemase genes, it is now important to know the specific gene produced by an EPC isolate in order to guide therapeutic decisions and avoid therapeutic impasse. Fortunately, cefiderocol is effective against all carbapenemases, making it the last therapeutic option in the treatment of difficult-to-treat GNB infections. Cefiderocol has demonstrated clinically relevant success rates and reduced renal risk (19).

In our study, plasmid-mediated quinolone resistance gene, *qnrS*, was seen in 71.0% of the clinical isolates (17/24) and *qnrB* in 21.0% (5/24) but no *qnrA* gene was observed. Presumably, the overuse of quinolones in our context has contributed to this high prevalence rate compared with similar studies conducted in hospitals in the subregion such as Côte d'Ivoire (20), Cameroon (21), Nigeria (6), and Togo (22). Our results on the prevalence of quinolone resistance genes thus confirm previous work carried out in Niger. Indeed, Illa et al., (23) had reported prevalence rates of 18.75% for the *qnrB* gene and 6.25% for *qnrS* gene in a study of antibiotic-resistant *Salmonella* and *Shigella*. Moumouni et al., (24) also reported rates of 9.5% (4/78) for *qnrA* gene, 26.2% (11/78) for *qnrB* and 64.3% (27/78) for *qnrS* in 2016.

Type 1 and 2 sulfa resistance genes (*sul1* and *sul2*) were observed in almost all the isolates, with *sul1* in 92.0% (22/24) and *sul2* in 50.0% (12/24) but no *sul3* was seen. These results are similar to those reported by Frank et al., (25) in Central Africa with *sul1* (67/78), sul2 (72/78) and both genes (62/78) and that reported by Dahmen et al., (26) in Tunisia with *sul1* (22/80), *sul2* (5/80) and both genes in 49/80.

Conclusion:

Our study, although preliminary, shows the importance of research into resistance genes for the various antibiotics we use routinely. Other rarely used antibiotics have been the subject of resistance when they should not have been. However, it is important to ensure the rational and appropriate use of medicines in general and antibiotics in particular. Antibiotic resistance is a real and growing problem that will reach dramatic proportions if we are not careful. In Africa, this problem is particularly acute, given the ubiquitous availability of low-quality and low-cost antibiotics. Unfortunately, the failure of healthcare workers to follow prescribing guidelines also contributes to the emergence of resistance.

In Niger, more widespread detection is needed to create a global map of antibiotic resistance. The environment and animals will not be left out of the "One Health" strategy.

Contributions of authors:

All authors contributed equally to the study design, methodology, manuscript preparation and approval of the original version submitted for publication.

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Conflict of interest:

No conflict of interest is declared.

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