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Resistance of *Klebsiella* to Imipenem by Production of Carbapenemase Gene bla_{IMP} at Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle, Ouagadougou, Burkina Faso

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Abstract

Objective: Class B carbapenemases are bacterial enzymes that catalyze the hydrolysis of β-lactam core antibiotics, except for monobactams. The objective of this study was to identify the carbapenemase gene bla_{IMP} in the genus *Klebsiella* at the Charles De Gaulle Pediatric University Hospital (CHUP-CDG) of Ouagadougou, Burkina Faso. **Methods:** The study involved 17 bacterial strains responsible for human infection and isolated from various biological samples during the period from 2009 to 2013. The strains were tested for antimicrobial susceptibility to cefotaxime, ceftazidime and imipenem using the Mueller-Hinton agar diffusion method. The carbapenemases resistance genes were detected by conventional PCR using specific primers at the molecular biology laboratory of CERBA/LABIOGENE, Ouagadougou, Burkina Faso. **Results:** The antibiotic susceptibility test showed high resistance of the 17 *Klebsiella* isolates tested to cephalosporins. A high cefotaxime-resistance rate (82.35%) and ceftazidime-resistance rate (88.23%) was found among the strains tested against 11.76% resistance rate for imipenem. Analysis of PCR products by gel electrophoresis revealed 4 strains (23.53%) with bla_{IMP}-type gene. **Conclusion:** *Klebsiella* is a well-known bacterium in clinical practice. The present study demonstrated the bla_{IMP}-type gene in cephalosporin-resistant strains of *Klebsiella* at CHUP-CDG. More effective monitoring and treatment solutions are needed to prevent the spread of these resistant strains.

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Keywords

Klebsiella, Resistance, bla_{IMP} Genes, β-lactam, Burkina Faso

1. Introduction

Klebsiella is a genus of flowering plants in the family Enterobacteriacea. It includes several species which are: *K. pneumoniae*, *K. oxytoca*, *K. ornithinolytica*, *K. planticola*, *K. terrigena*, *K. granulomatis*, *K. variicola*, *K. singaporensis* [1]. The species *K. pneumoniae* is often implicated in human infections. It is an opportunistic agent of community and nosocomial infections such as urinary tract infections, respiratory tract infections and wound infections. *K. pneumoniae* is a commensal bacterium of the gastrointestinal and respiratory tracts. It is also present in the environment [2]. *Klebsiella oxytoca* is an opportunistic pathogen in humans and is increasingly associated to nosocomial infections, particularly in immunocompromised individuals [3]. *Klebsiella* species are naturally resistant to penicillin such as ampicillin, ticarcillin and piperacillin. Wild strains are susceptible to antibiotics active on gram-negative bacteria such as aminoglycosides, fluoroquinolones, sulfonamides, fosfomycin, colistin, furans, cephalosporins and carbapenems [4]. However, the emergence of β-lactamases, enzymes capable of hydrolyzing β-lactams, is increasingly described in clinical isolates from *Klebsiella* which is a public health concern. [3] According to Ambler's classification, β-lactamases are subdivided into four groups (A, B, C and D) based on their amino acid sequences [5].

KPC Class A carbapenemases and zinc-dependent Class B metallo-β-lactamases (MBL), primarily VIM, IMP and NDM metallo-β-lactamases, are the most clinically important carbapenemases in *Enterobacteriacea* [5] [6]. These β-lactamases hydrolyze penicillins, cephalosporins and carbapenems, although significant variations in hydrolytic efficiency exist even between enzymes of the same type [7]. The emergence of carbapenemases-producing *Enterobacteriaceae*, particularly *Klebsiella*, which produces KPC, VIM, IMP and NDM carbapenemases, is a public health concern [7]. Carbapenemase genes have been described in several epidemiological studies in Europe, North America and Asia [8] [9]. In Africa, there is little data available on carbapenemase genes. A previous study reported the presence of carbapenemase-producing bacteria at rates of 74% in North Africa; 12% in Southern Africa; 90% in South Africa; 8% in West Africa (7/83) and 6% in East Africa [10]. In Burkina Faso, a study on germ resistance revealed the presence of Verona integrin-encoded metallo-β-lactamase (VIM) and imipenemase (IMP-2) reaching a rate of 40% each [11]. However, no information is available on the different types of carbapenemase genes harbored by *Klebsiella* species.

The main objective of this study was to characterize the IMP-type carbapenemase gene carried by *Klebsiella* species, isolated between 2009 and 2013 at the Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle, Ouagadougou.

2. Material and Methods

2.1. Bacterial Strains

Bacterial isolates were collected consecutively between 2009 and 2013 from children at the CHUP-CDG of Ouagadougou, Burkina Faso [12]. They were isolated from various clinical specimens such as urine, pus, and CSF from diverse units of the hospital and conserved in the biobank of CERBA/LABIOGENE. Once identified, the isolates were preserved at −80˚C in Tryptic Soy Broth containing glycerol 15% (v/v). From June to September 2021, the 17 *Klebsiella* strains were selected from the biobank for antibiogram testing and screening for the bla_{IMP} gene.

2.2. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test was performed by the disk diffusion method on Mueller Hinton (MH) agar using pure *Klebsiella* colonies according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [4]. The antibiotic disks used were Cefotaxime, Ceftazidime, and Imipenem.

2.3. DNA Extraction

The boiling method was used to extract DNA [13]. The strains were reactivated by culture on the MH medium for 18 to 24 h and then in liquid LB medium. The bacterial suspensions were then immersed in a water bath at 100˚C for 15 minutes to release the bacteria's genetic material. After that, a centrifugation of 10 min at 12,000 rpm was performed and the supernatant containing the released DNA was transferred to a new Eppendorf tube. Total DNA quantification and DNA purity based on absorbance ratios A260/A280 and A260/A230 were determined for each sample using NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

2.4. Antibiotic-Resistant Gene Amplification

The Bla_{IMP} genes were detected by PCR with the following specific primers: IMPF; 5'CATGGTGTGGTGCTTGT3' and IMP-R; 5'ATAATTTGGCGGACTTTGGC3', with expected amplicon size 488 bp [14]. The PCR amplification was performed in 20 µl volumes containing 4 µL of GREEN PCR Master Mix 5X, 0.5 µL of each primer, 14 µL of sterile PCR water and 1 µL of DNA template in a Gene Amp 9700 PCR System thermocycler (Applied Biosystems, California, USA). The PCR conditions consisted of denaturation at 96˚C for 5 min, then 30 cycles of denaturation for 30 s at 95˚C, annealing for 30 s at 54˚C and extension for 30 s at 72˚C following by a final extension for 7 min at 72˚C.

2.5. Electrophoresis

PCR products were loaded on a 1.5% agarose gel with 0.1% ethidium bromide in 1X Tris-acetate EDTA (TAE) buffer, separated for 25 min at 100 millivolts, and

visualized under ultraviolet light using GeneFlash (Syngene, Bio-Imaging, UK). A 100 bp DNA ladder was used to visualize fragments size. PCR mix with no DNA template was included as a negative control.

2.6. Statistical Analysis

The collected data was entered into Microsoft excel spreadsheet, cleaned, and imported into STATA software package v.14.0 (Stata Corporation, College Station, TX) for analysis. Data were described as percentage (%) and frequency of occurrence for categorical variables.

2.7. Ethical Considerations

The institutional ethic committee of CERBA/LABIOGENE reviewed and approved the study protocol.

3. Results

3.1. Bacterial Isolates

From 2009 to 2013, a total of 17 *Klebsiella* isolates were collected from the different wards of CHUP-CDG, Ouagadougou, Burkina Faso. Urine provided the majority of the *Klebsiella* clinical isolates (n = 10, 58.82%), followed by pus (n = 6, 35.30%) and cerebrospinal fluid (n = 1, 5.88%).

3.2. Antimicrobial Susceptibility Testing

A total of 17 *Klebsiella* isolates from clinical specimens were tested for antimicrobial susceptibility to cefotaxime (CTX), ceftazidime (CAZ) and imipenem. The results revealed a high cefotaxime (14/17; 82.35%) and ceftazidime (15/17, 88.23%) resistance rate against 11.76% (4/17) resistance rate for imipenem. It is noteworthy that 8 out of 10 *Klebsiella pneumoniae* and all 4 *K. oxytoca* were resistant to CTX and CAZ (**Table 1**).

3.3. Molecular Characterization of bla_{IMP} Genes

The bla_{IMP} metallo-β-lactamases genes were sought by PCR in 17 *Klebsiella* isolates from CHUP-CDG and 3 (4/17 or 23.53%) of them yielded the amplicon of bla_{IMP} gene (**Figure 1**). Among these 4 bla_{IMP}-carrying strains, 3 were *Klebsiella oxytoca* and 1 was *Klebsiella pneumoniae* (**Table 2**).

4. Discussion

Except for aztreonam, MBLs may confer resistance to carbapenems and all other β-lactam [15]. MBL types of VIM, IMP and NDM are the most common in the world [16]. These enzymes are mainly found in gram-negative bacilli. MBL IMP has been reported primarily in *P. aeruginosa*, *Acinetobacter spp* and several species of Enterobacteriaceae, including *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, *E. cloacae* and *Citrobacter spp* [17]. Bacteria carrying the bla_{IMP} gene are referred to as carbapenem-resistant Enterobacteriaceae (CRE). The present study was

Table 1. Distribution of resistance by species.

Species	Antimicrobial N (%)		
	CTX	CAZ	IMP
Klebsiella pneumoniae	8 (43.06%)	8 (43.06%)	1 (5.88%)
Klebsiella oxytoca	4 (23.53%)	4 (23.53%)	1 (5.88%)
Klebsiella sp.	2 (11.76%)	3 (17.64%)	0 (0.00%)
Total	14 (82.35%)	15 (88.23%)	2 (11.76%)

Abbreviations: CTX, Cefotaxime; CAZ, Ceftazidime; IMP, Imipenem.

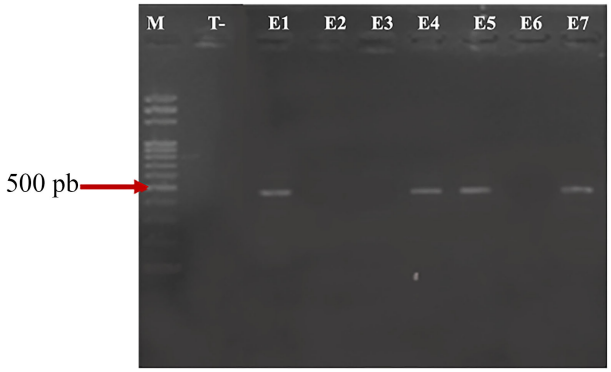

Figure 1. Electrophoretic profile of the *bla*$_{IMP}$ gene. M1: Molecular weight marker (100 pb DNA Ladder). T-: Negative control. The numbers E1-E7 represent the samples. The direction of migration of electrophoresis is from top to bottom.

Table 2. Distribution of the IMP gene by species.

Species	*Bla*$_{IMP}$ gene
Klebsiella pneumonia	3 (23.53%)
Klebsiella oxytoca	1 (11.76%)
Klebsiella sp.	0 (00.00%)
Total	3 (17.64%)

focused on the identification of carbapenem-resistant *Klebsiella* collected from 2008 to 2013. The antimicrobial susceptibility test revealed significant resistance to third generation cephalosporins (C3G). The resistance rate was 82.35% for cefotaxime (CTX) and 88.23% for ceftazidime (CAZ). C3G resistance has been reported in Benin [18] (97.5% for CAZ and CRO), Togo [19] (97.28% for CAZ, 97.16% for CRO and 100% for CTX) and Burkina Faso [20] (76.6% for CTX, 75% for CRO, 58.3% for CAZ). Resistance to third generation cephalosporins has been reported in *Klebsiella pneumoniae* strains in Bangladesh [21] (ceftriaxone 72.8%, and ceftazidime 75.3%) in Iran [22] (ceftriaxone 73% and ceftazidime 72%). The high rate of resistance to third generation cephalosporins observed in this study could be due to the presence of certain resistance genes. These genes are located on the bacterial chromosome or on mobile genetic ele-

ments such as plasmids or transposons that can be transferred from one strain to another of the same species, but also between two closely related species [23] [24].

In our study, carbapenem (35.28%) was weakly resistant, as were those in Bangladesh [21] (meropenem for 44.7%) and Iran (meropenem 43.4%). It has been very sensitive in India [25] (100% for imipenem), Ghana [26] (100% for meropenem) and Iran [27] (imipenem for 58.2%; meropenem for 64.3%; doripenem for 64.3%; ertapenem for 66.4%). These results show why carbapenems are used in the treatment of infections by multidrug-resistant bacteria. The rate of imipenem resistance in this study could be due to chromosomal mechanisms by porin alteration, the combination of resistance mechanisms (*B*LSE and/or cephalosporinase associated with loss of membrane permeability) or the production of carbapenemases [28].

The spread of bla_{IMP} gene-mediated resistance is a concerning issue in healthcare settings and communities worldwide. It can lead to difficult-to-treat infections and can have serious implications for public health, as it may result in the limited effectiveness of available antibiotics [29]. To combat this, it is essential to monitor and track the prevalence of bla_{IMP} gene in bacterial strains to implement appropriate infection control measures and preserve the efficacy of existing antibiotic treatments [30]. In this study, we obtained 4 strains positive (23.53%) for IMP-type metallo-β-lactamases including 3 strains of *Klebsiella pneumoniae* and 01 strain of *Klebsiella oxytoca*. The presence of IMP-type metallo-β-lactamase has been reported in *Klebsiella pneumoniae* in several countries such as Iran [27] [31], the United States [32], China [33] [34], Brazil [35] and Japan [36]. The presence of the IMP-type metallo-β-lactamase has also been detected in China [37] and Australia [38]. The prevalence of the IMP gene has been reported in Gram-negative bacilli in Tanzania [39], Egypt [40] [41] and Sudan [42] at 12%; 4.2%; 20%; and 26.4%, respectively. All this testifies to the global distribution of IMP-type carbapenemase-producing *Enterobacteriaceae* and the very high variability of their prevalence depending on the country. The presence of this gene in our study could be explained by the fact that this gene is usually located on mobile genetic elements (plasmids, transposons, integrons) allowing for rapid dissemination worldwide [28] [43]. The presence of this gene would explain the multidrug resistance of strains carrying it. One of the limitations of this study is that it focused on bacterial strains collected between 2009 and 2013. However, it provides an overview of antimicrobial resistance of clinical strains of Klebsiella in Burkina Faso. Research and development of new antimicrobial agents and strategies are crucial in addressing the challenges posed by bla_{IMP}-mediated resistance and other antibiotic resistance mechanisms.

5. Conclusion

Altogether the present study showed high resistance of *Klebsiella* to third generation cephalosporins and a low resistance rate to imipenem. We were also able

to identify the *bla*$_{IMP}$ gene. Multidrug resistance in Enterobacteriaceae raises public health concerns. Surveillance measures must be taken to prevent the emergence of carbapenemase-producing bacteria through enzyme production.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Podschun, R. and Ullmann, U. (1998) *Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. *Clinical Microbiology Reviews*, **11**, 589-603. https://doi.org/10.1128/CMR.11.4.589

[2] Herridge, W.P., Shibu, P., O'Shea, J., Brook, T.C. and Hoyles, L. (2020) Bacteriophages of *Klebsiella* spp., Their Diversity and Potential Therapeutic Uses. *Journal of Medical Microbiology*, **19**, 176-194.

[3] Broberg, C.A., Palacios, M. and Miller, V.L. (2014) *Klebsiella*: A Long way to Go towards Understanding This Enigmatic Jet-Setter. *F*1000*Prime Reports*, **6**, Article No. 64. https://doi.org/10.12703/P6-64

[4] CA-SFM-EUCAST (2021) Comité de l'antibiogramme de la Société Française de Microbiologie. https://www.sfm-microbiologie.org/wp-content/uploads/2021/04/CASFM2021__V1.0.AVRIL_2021.pdf

[5] Hall, B.G. and Barlow, M. (2005) Revised Ambler Classification of β-Lactamases. *Journal of Antimicrobial Chemotherapy*, **55**, 1050-1051. https://doi.org/10.1093/jac/dki130

[6] Bush, K. and Jacoby, G.A. (2010) Updated Functional Classification of β-Lactamases. *Antimicrobial Agents and Chemotherapy*, **54**, 969-976. https://doi.org/10.1128/AAC.01009-09

[7] Tzouvelekis, L.S., Markogiannakis, A., Psichogiou, M., Tassios, P.T. and Daikos, G.L. (2012) Carbapenemases in *Klebsiella pneumoniae* and Other *Enterobacteriaceae*: An Evolving Crisis of Global Dimensions. *Clinical Microbiology Reviews*, **25**, 682-707. https://doi.org/10.1128/CMR.05035-11

[8] Cantón, R., Akóva, M., Carmeli, Y., Giske, C.G., Glupczynski, Y., Gniadkowski, M., Livermore, D.M., Miriagou, V., Naas, T., Rossolini, G.M., Samuelsen, Ø., Seifert, H., Woodford, N. and Nordmann, P. (2012) Rapid Evolution and Spread of Carbapenemases among *Enterobacteriaceae* in Europe. *Clinical Microbiology and Infection*, **18**, 413-431. https://doi.org/10.1111/j.1469-0691.2012.03821.x

[9] Lascols, C., Peirano, G., Hackel, M., Laupland, K.B. and Pitout, J.D.D. (2013) Surveillance and Molecular Epidemiology of *Klebsiella pneumoniae* Isolates That Produce Carbapenemases: First Report of OXA-48-Like Enzymes in North America. *Antimicrobial Agents and Chemotherapy*, **57**, 130-136. https://doi.org/10.1128/AAC.01686-12

[10] Manenzhe, R.I., Zar, H.J., Nicol, M.P. and Kaba, M. (2015) The Spread of Carbapenemase-Producing Bacteria in Africa: A Systematic Review. *Journal of Antimicrobial Chemotherapy*, **70**, 23-40. https://doi.org/10.1093/jac/dku356

[11] Dembélé, R., Soulama, I., Kaboré, W.A.D., Konaté, A., Kagambèga, A., N'Golo, D.C., Traoré, O., Seck, A., Traoré, A.S., Guessennd, N.K., Gassama-Sow, A. and Barro, N. (2021) Molecular Characterization of Carbapenemase-Producing *Enterobacteralesin* Children with Diarrhea in Rural Burkina Faso. *Journal of Drug Delivery and Therapeutics*, **11**, 84-92. https://doi.org/10.22270/jddt.v11i1.4513

[12] Mètuor, A. (2014) Caractérisations moléculaire et cinétique des types de β-lactamases à spectre élargi (BLSE) de souches bactériennes collectées au Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle (CHUP-CDG) de Ouagadougou. Université de Ouagadougou, Ouagadougou.

[13] Mètuor, D.A., Tiemtoré, R.Y.W.-K., Bangré, Y.A., Zohoncon, T.M., Sougué, S., Zongo, J.K. and Simporé, J. (2019) Detection of Multidrug-Resistant Enterobacteria Simultaneously Producing Extended-Spectrum Î²-Lactamases of the PER and GES Types Isolated at Saint Camille Hospital Center, Ouagadougou, Burkina Faso. *African Journal of Microbiology Research*, **13**, 414-420. https://doi.org/10.5897/AJMR2019.9147

[14] Huang, S., Dai, W., Sun, S., Zhang, X. and Zhang, L. (2012) Prevalence of Plasmid-Mediated Quinolone Resistance and Aminoglycoside Resistance Determinants among Carbapeneme Non-Susceptible *Enterobacter cloacae*. *PLOS ONE*, **7**, e47636. https://doi.org/10.1371/journal.pone.0047636

[15] Walsh, T.R., Toleman, M.A., Poirel, L. and Nordmann, P. (2005) Metallo-β-Lactamases: The Quiet before the Storm? *Clinical Microbiology Reviews*, **18**, 306-325. https://doi.org/10.1128/CMR.18.2.306-325.2005

[16] Pitout, J.D.D., Nordmann, P. and Poirel, L. (2015) Carbapenemase-Producing *Klebsiella pneumoniae*, a Key Pathogen Set for Global Nosocomial Dominance. *Antimicrobial Agents and Chemotherapy*, **59**, 5873-5884. https://doi.org/10.1128/AAC.01019-15

[17] Martinez, J.L. (2014) General Principles of Antibiotic Resistance in Bacteria. *Drug Discovery Today*, **11**, 33-39. https://doi.org/10.1016/j.ddtec.2014.02.001

[18] Ahoyo, A.T., Baba-Moussa, L., Anago, A.E., Avogbe, P., Missihoun, T.D., Loko, F., Prévost, G., Sanni, A. and Dramane, K. (2007) Incidence d'infections liées à *Escherichia coli* producteur de bêta lactamase à spectre élargi au Centre hospitalier départemental du Zou et Collines au Bénin. *Médecine et Maladies Infectieuses*, **37**, 746-752. https://doi.org/10.1016/j.medmal.2007.03.004

[19] Toudji, A.G., Djeri, B., Karou, S.D., Tigossou, S., Ameyapoh, Y. and De Souza, C. (2017) Prévalence des souches d'entérobactéries productrices de bêta-lactamases à spectre élargi isolées au Togo et de leur sensibilité aux antibiotiques. *International Journal of Biological and Chemical Sciences*, **11**, 1165-1177. https://doi.org/10.4314/ijbcs.v11i3.19

[20] Mètuor-Dabiré, A., Sougué, S., Tiemtoré, R.Y.W.-K., Zohoncon, T.M., Bangré, Y.A., Ouédraogo, P., Kabré, E. and Simporé, J. (2019) Coexistence between (TOHO-Type and BES-Type) Extended-Spectrum Î²-Lactamase Genes of Identified Enterobacteria at Saint Camille Hospital, Ouagadougou, West Africa. *International Journal of Genetics and Molecular Biology*, **11**, 34-40. https://doi.org/10.5897/IJGMB2019.0181

[21] Aminul, P., Anwar, S., Molla, M.M.A. and Miah, M.R.A. (2021) Evaluation of Antibiotic Resistance Patterns in Clinical Isolates of *Klebsiella pneumoniae* in Bangla-

desh. *Biosafety and Health*, **3**, 301-306. https://doi.org/10.1016/j.bsheal.2021.11.001

[22] Barakzahi, M., Hormozi, B., Rashki, A. and Rashki Ghalehnoo, Z. (2014) Prevalence of Extended Spectrum β-Lactamase in *Klebsiella pneumonia* Isolates in a Teaching Hospital of Zahedan City, Iran. *Avicenna Journal of Clinical Microbiology and Infection*, **1**, Article No. 22934. https://doi.org/10.17795/ajcmi-22934

[23] Harbottle, H., Thakur, S., Zhao, S. and White, D.G. (2006) Genetics of Antimicrobial Resistance. *Animal Biotechnology*, **17**, 111-124. https://doi.org/10.1080/10495390600957092

[24] Muylaert, A. and Mainil, J. (2013) Résistance bactériennes aux antibiotiques, les mécanismes et leur "contagiosité". *Annales de Médecine Vétérinaire*, **156**, 109-123.

[25] Shashwati, N., Kiran, T. and Dhanvijay, A.G. (2014) Study of Extended Spectrum β-Lactamase Producing *Enterobacteriaceae* and Antibiotic Coresistance in a Tertiary Care Teaching Hospital. *Journal of Natural Science, Biology and Medicine*, **5**, 30-35.

[26] Teklu, D.S., Negeri, A.A., Legese, M.H., Bedada, T.L., Woldemariam, H.K. and Tullu, K.D. (2019) Extended-Spectrum Beta-Lactamase Production and Multi-Drug Resistance among *Enterobacteriaceae* Isolated in Addis Ababa, Ethiopia. *Antimicrobial Resistance & Infection Control*, **8**, Article No. 39. https://doi.org/10.1186/s13756-019-0488-4

[27] Alizadeh, H., Khodavandi, A., Alizadeh, F. and Bahador, N. (2021) Molecular Characteristics of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates Producing *bla*$_{VIM}$, *bla*$_{NDM}$, and *bla*$_{IMP}$ in Clinical Centers in Isfahan, Iran. *Jundishapur Journal of Microbiology*, **14**, e114473. https://doi.org/10.5812/jjm.114473

[28] Nordmann, P., Naas, T. and Poirel, L. (2011) Global Spread of Carbapenemase-Producing *Enterobacteriaceae*. *Emerging Infectious Diseases*, **17**, 1791-1798. https://doi.org/10.3201/eid1710.110655

[29] Gao, H., Liu, Y., Wang, R., Wang, Q., Jin, L. and Wang, H. (2020) The Transferability and Evolution of NDM-1 and KPC-2 Co-Producing *Klebsiella pneumoniae* from Clinical Settings. *EBioMedicine*, **51**, Article ID: 102599. https://doi.org/10.1016/j.ebiom.2019.102599

[30] Munita, J.M. and Arias, C.A. (2016) Mechanisms of Antibiotic Resistance. *Microbiology Spectrum*, **4**. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

[31] Khodadadian, R., Rahdar, H.A., Javadi, A., Safari, M. and Khorshidi, A. (2018) Detection of VIM-1 and IMP-1 Genes in *Klebsiella pneumoniae* and Relationship with Biofilm Formation. *Microbial Pathogenesis*, **115**, 25-30. https://doi.org/10.1016/j.micpath.2017.12.036

[32] Limbago, B.M., Rasheed, J.K. anderson, K.F., Zhu, W., Kitchel, B., Watz, N., Munro, S., Gans, H., Banaei, N. and Kallen, A.J. (2011) IMP-Producing Carbapenem-Resistant *Klebsiella pneumoniae* in the United States. *Journal of Clinical Microbiology*, **49**, 4239-4245. https://doi.org/10.1128/JCM.05297-11

[33] Han, R., Shi, Q., Wu, S., Yin, D., Peng, M., Dong, D., Zheng, Y., Guo, Y., Zhang, R. and Hu, F. (2020) Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among Carbapenem-Resistant *Enterobacteriaceae* Isolated from Adult and Children Patients in China. *Frontiers in Cellular and Infection Microbiology*, **10**, Article 314. https://doi.org/10.3389/fcimb.2020.00314

[34] Xu, J., Lin, W., Chen, Y. and He, F. (2020) Characterization of an IMP-4-Producing *Klebsiella pneumoniae* ST1873 Strain Recovered from an Infant with a Bloodstream Infection in China. *Infection and Drug Resistance*, **13**, 773-779. https://doi.org/10.2147/IDR.S247341

[35] Lincopan, N., McCulloch, J.A., Reinert, C., Cassettari, V.C., Gales, A.C. and Mamizuka, E.M. (2005) First Isolation of Metallo-β-Lactamase-Producing Multiresistant *Klebsiella pneumoniae* from a Patient in Brazil. *Journal of Clinical Microbiology*, **43**, 516-519. https://doi.org/10.1128/JCM.43.1.516-519.2005

[36] Fukigai, S., Alba, J., Kimura, S., Iida, T., Nishikura, N., Ishii, Y. and Yamaguchi, K. (2007) Nosocomial Outbreak of Genetically Related IMP-1 β-Lactamase-Producing *Klebsiella pneumoniae* in a General Hospital in Japan. *International Journal of Antimicrobial Agents*, **29**, 306-310. https://doi.org/10.1016/j.ijantimicag.2006.10.011

[37] Wang, J., Yuan, M., Chen, H., Chen, X., Jia, Y., Zhu, X., Bai, L., Bai, X., Fanning, S., Lu, J. and Li, J. (2017) First Report of *Klebsiella oxytoca* Strain Simultaneously Producing NDM-1, IMP-4, and KPC-2 Carbapenemases. *Antimicrobial Agents and Chemotherapy*, **61**, e00877-17. https://doi.org/10.1128/AAC.00877-17

[38] Stewart, J., Judd, L.M., Jenney, A., Holt, K.E., Wyres, K.L. and Hawkey, J. (2022) Epidemiology and Genomic Analysis of *Klebsiella oxytoca* from a Single Hospital Network in Australia. *BMC Infectious Diseases*, **22**, Article No. 704. https://doi.org/10.1186/s12879-022-07687-7

[39] Mushi, M.F., Mshana, S.E., Imirzalioglu, C. and Bwanga, F. (2014) Carbapenemase Genes among Multidrug Resistant Gram Negative Clinical Isolates from a Tertiary Hospital in Mwanza, Tanzania. *BioMed Research International*, **2014**, Article ID: 303104. https://doi.org/10.1155/2014/303104

[40] Zafer, M.M., Al-Agamy, M.H., El-Mahallawy, H.A., Amin, M.A. and Ashour, M.S.E.-D. (2014) Antimicrobial Resistance Pattern and Their Beta-Lactamase Encoding Genes among *Pseudomonas aeruginosa* Strains Isolated from Cancer Patients. *BioMed Research International*, **2014**, Article ID: 101635. https://doi.org/10.1155/2014/101635

[41] Aghamiri, S., Amirmozafari, N., Fallah Mehrabadi, J., Fouladtan, B. and Samadi Kafil, H. (2014) Antibiotic Resistance Pattern and Evaluation of Metallo-Beta Lactamase Genes Including *bla*-IMP and *bla*-VIM Types in *Pseudomonas aeruginosa* Isolated from Patients in Tehran Hospitals. *International Scholarly Research Notices*, **2014**, Article ID: 941507. https://doi.org/10.1155/2014/941507

[42] Adam, M.A. and Elhag, W.I. (2018) Prevalence of Metallo-β-Lactamase Acquired Genes among Carbapenems Susceptible and Resistant Gram-Negative Clinical Isolates Using Multiplex PCR, Khartoum Hospitals, Khartoum Sudan. *BMC Infectious Diseases*, **18**, Article No. 668. https://doi.org/10.1186/s12879-018-3581-z

[43] Nordmann, P. (2014) Carbapenemase-Producing *Enterobacteriaceae*: Overview of a Major Public Health Challenge. *Médecine et Maladies Infectieuses*, **44**, 51-56. https://doi.org/10.1016/j.medmal.2013.11.007