ECRONICON OPEN ACCESS

EC CLINICAL AND MEDICAL CASE REPORTS

Research Article

Epidemiological and Bacteriological Profile of Carbapenemase Producing *Enterobacteriaceae* in the Laboratory of Microbiology Department at the Hassan II University Hospital in Fez

Kadiri M[1,2,3]*, Kouara S[1,2,3], Mahmoud M[1,2,3] and Yahyaoui G[1,2,3]

[1]*Bacteriology Department of the Central Laboratory for Medical Biological Analysis, Hassan II University Hospital, Fez, Morocco*

[2]*Faculty of Medicine and Pharmacy, Fez, Morocco*

[3]*Sidi Mohammed Benabdellah University, Fez, Morocco*

***Corresponding Author:** Kadiri M, Bacteriology Department of the Central Laboratory for Medical Biological Analysis, Hassan II University Hospital, Fez, Morocco.

Received: July 09, 2024; **Published:** July 24, 2024

Abstract

Carbapenemase producing *Enterobacteriaceae* are a family of gram-negative bacteria found in the gastrointestinal tract, responsible for the production of carbapenemase enzymes, which render carbapenems and several other classes of antibiotics inactive. The emergence of these bacteria poses a threat to public health, particularly in the hospital environment, given their resistance profile and the significant riwt types of samples received during this period, notably urinary, respiratory, blood samples from blood culture bottles, pus, puncture fluids and catheters.

Each sample was processed in several stages:

- Direct examination.
- Culture for 24 to 48 hours, followed by direct examination with GRAM staining for any cultures that had grown.
- Identification of the bacterial species using the Api 20 E gallery or the bactec BD automated system, in accordance with CASFM/EUCAST recommendations.
- An antibiogram selected and performed according to the bacterial species identified.

In the event of a carbapenem-resistant bacterial profile, screening for carbapenemases is necessary, using a rapid immunochromatography test.

Of the 9205 strains of *Enterobacteriaceae* identified in the above-mentioned samples, 110 were found to produce carbapenemases, i.e. 1%. This percentage still seems very low in comparison with other studies, which we'll be detailing as we go along.

The most clinically and epidemiologically significant carbapenemases are *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-betalactamase (NDM-1) and Oxacillinase (OXA-48). Several countries not considered endemic have experienced recurrent epidemics of EPCs, which can be exported to neighboring countries. Most carbapenemase-producing *Enterobacteriaceae* are multiresistant to other antibiotic classes, which limits their therapeutic potential. In practical terms, the antibiotics of choice are colimycin, tigecycline, fosfomycin and a few quinolones. Mortality seems to be even lower when treatment combines two antibiotics to which the strain remains sensitive.

Keywords: *Enterobacteriaceae; Carbapenemases; Resistance; OXA-NDM*

Introduction

Antibiotic resistance in enterobacteria in general, and in carbapenemase-producing enterobacteria in particular, has become a major concern requiring rapid, organized management, given the high morbidity and mortality associated with these infections [1]. The majority of carbapenemase-producing *Enterobacteriaceae* carry resistance genes to other antibiotic classes, making them resistant not only to carbapenems, but also to other antibiotics, and are easily transmitted between *Enterobacteriaceae* due to their plasmid localization [2]. The optimal treatment of infections caused by carbapenemase *Enterobacteriaceae* is not yet well defined, hence the importance of an effective prevention and screening strategy to control the spread of these bacteria within the hospital setting. The most clinically and epidemiologically important carbapenemase types are KPC (*Klebsiella pneumoniae* carbapenemase), NDM-1 (New Delhi metallo-betalactamase) and OXA-48 (Oxacillinase). According to several studies, *Klebsiella pneumoniae* remains the most carbapenemase-producing bacterial strain [3]. The spread of carbapenemase bacteria is of worldwide interest, particularly in hospitals, where antibiotic use remains massive [4,5].

Our study will focus on the epidemiological, clinical and bacteriological profile of carbapenemase-producing *Enterobacteriaceae*, the main aim of which is to assess the resistance profile of this type of bacteria, as well as the prognosis of our patients before and after appropriate antibiotic therapy.

Materials and Methods

This is a retrospective study spread over a two-year period, from October 2021 to 2023, carried out in the bacteriology department of the central laboratory for medical biological analysis at the Hassan II University Hospital in Fez.

This study was carried out on different types of samples received during this period, notably urinary, respiratory, blood samples from blood culture bottles, pus, puncture fluids and catheters.

Each sample was processed in several stages:

- Direct examination for cytology and bacteria.
- Culture for 24 to 48 hours, followed by direct examination with GRAM stain for any cultures that had grown, in order to classify the bacteria found.
- Identification of the bacterial species based on different methods chosen according to each suspected bacterial species: biochemical, enzymatic, immunological.
- An antibiogram selected and performed according to the bacterial species identified.

Any strain with reduced sensitivity to at least one of the carbapenems is considered to be suspected of being resistant to carbapenems. Detection of EPCs by simple phenotypic tests is not always easy, as the level of resistance to carbapenems is variable and may sometimes be at the limit of the sensitivity threshold. Ertapenem is the carbapenem with the best sensitivity for EPC detection. Any strain with decreased sensitivity to ertapenem: MIC ≥ 0.5 mg/L or inhibition diameter < 28 mm on 10 µg disks by agar diffusion test is suspected of being an EPC. Furthermore, in some cases, the sensitivity of detection of carbapenemase production can be improved by using at least 2 different carbapenems (e.g. imipenem and ertapenem) (Figure 1).

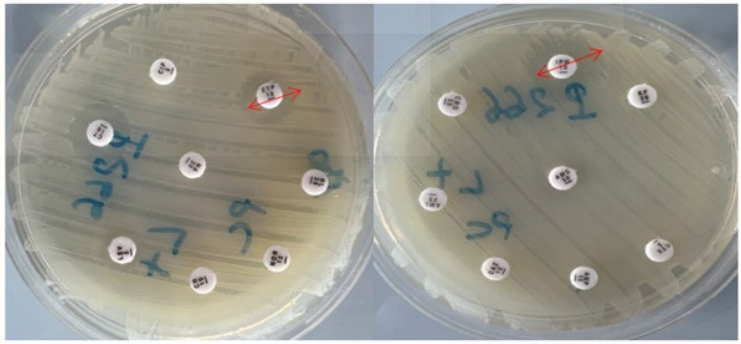

Figure 1: *Antibiogram of an EPC strain showing resistance to carbapenems (Ertapenem and Imipenem) with an inhibition diameter of ertapenem < 28 mm.*

Bacteriology Department of the Central Laboratory, Hassan II University Hospital, Fez.

Immunochromatographic rapid test

When faced with a carbapenem-resistant bacteriological profile, carbapenemase screening is necessary, using a rapid immunochromatography test (Figure 2) for positive diagnosis of the carbapenemase and its type.

Figure 2: *Rapid carbapenemase test positive for NDM.*

Bacteriology Department of the Central Laboratory, Hassan II University Hospital, Fez.

Modified Hodge test

This is a qualitative phenotypic test for the detection of carbapenemase-producing *Enterobacteriaceae*. A preparation of a 0.5 McFarland dilution of susceptible *Escherichia coli* in 5 ml of broth or saline solution. A 1:10 dilution spread as a lawn on a Mueller Hinton

agar plate. A 10 µg meropenem or ertapenem sensitivity disc is placed in the center of the test area. The test organism is streaked in a straight line from the edge of the disc to the edge of the plate. The positive and negative quality controls are spread in the same way, using one carbapenemase-producing and one sensitive strain of enterobacteria. Incubation at 35 ± 2°C for 16 to 24 hours.

After 24 hours, the Hodge test is positive if it shows a clover-leaf-shaped indentation of the test strain growing along the growth streak in the diffusion zone of the disc. The test is negative if it shows no growth of the test strain along the growth streak within the disc diffusion (Figure 3).

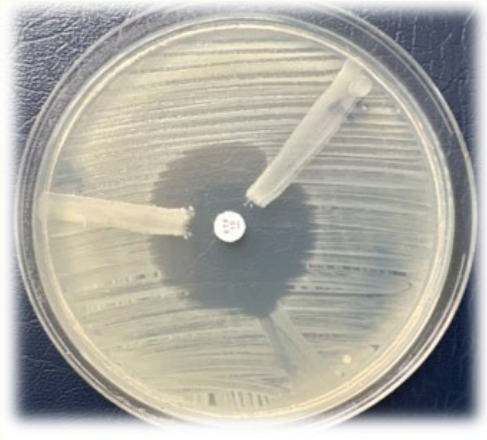

Figure 3: *Modified Hodge test.*

Bacteriology Department of the Central Laboratory, Hassan II University Hospital, Fez.

SES: Sensitive Escherichia coli Strain.

ECCS: Escherichia coli Carbapenemase Control Strain.

CTS: Carbapenemase Test Strain.

Of the 9205 strains of *Enterobacteriaceae* identified on the above samples, 110 were found to be carbapenemase producers, i.e. 1%. *Klebsiella pneumoniae* was the most dominant species at 55%, followed by *Escherichia coli* at 32%, *Proteus mirabilis* and *Enterobacter cloacae* at 7% and 6% respectively. Of the 110 strains isolated, the carbapenemase types encountered were NDM, present at 36%, OXA 48 at 35% or a combination of the two at 28%. Pus was the sample from which we isolated the highest number of carba-resistant enterobacteria, at 52%, followed by urine at 27%. With the exception of strains of *Proteus mirabilis*, which is naturally resistant to colimycin, all the rest were sensitive to this antibiotic, and only 27% of strains retained sensitivity to aminoglycosides.

Results

Epidemiology

Of the 9205 enterobacteria isolated, carbapenemase-producing bacteria (CPE) accounted for 110 or 1% (Figure 4).

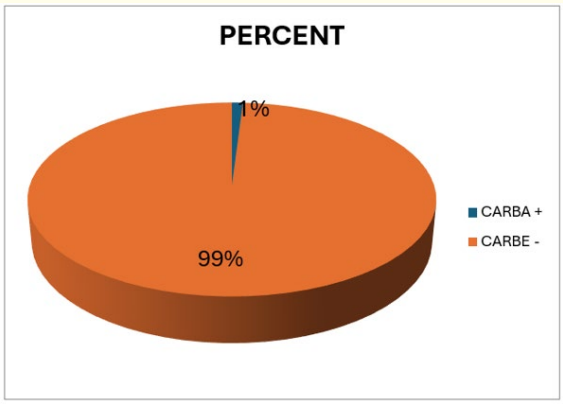

Figure 4: *Frequency of carbapenemase-producing Enterobacteriaceae.*

In 2021, 19 strains of carbapenemase-producing *Enterobacteriaceae* were isolated, representing a percentage of 17%. From 2022 onwards, the number of EPCs rose sharply to 45, a percentage of 41%, and remained relatively stable in 2023 at 42% (Figure 5).

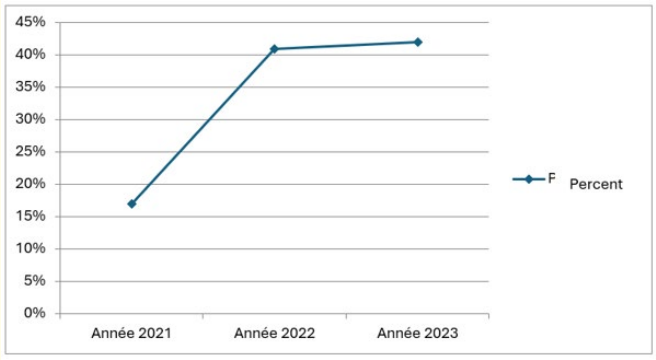

Figure 5: *Annual incidence of EPCs.*

Hospitalization

Reasons for hospitalization

Eight percent (8%) of our patients identified as having carbapenemase bacterial infections were operated on, having first been admitted to the operating theatre and then to the intensive care unit, representing the most common reason for admission. The remaining 54% of our patients were admitted for a variety of much less frequent symptoms or pathologies. It should be noted that these were all patients who had been through the emergency department before being admitted, or who had been previously hospitalized.

Inpatient department

EPCs were identified in the various departments of our university hospital. A large percentage of infected patients were hospitalized in intensive care units, accounting for 41%. In medical wards, 30% of EPCs were detected, 21% in surgical wards and only 08% in emergency wards (Figure 6).

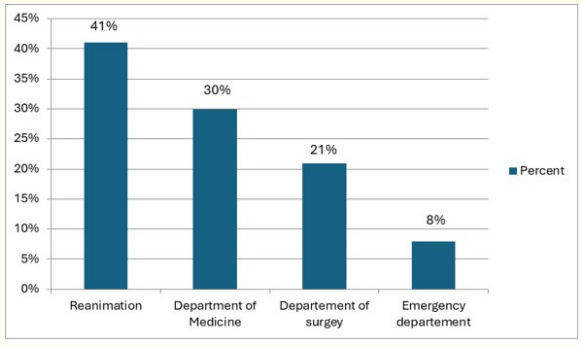

Figure 6: *Percentage of EPCs isolated according to hospitalization service.*

Bacteriological diagnosis of EPC

Nature of samples tested positive for EPC

EPCs were predominant in pus samples (51%), followed by ECBU (27%), blood cultures (13%), catheters (5%), protected distal samples (3%) and lumbar punctures (1%) (Figure 7).

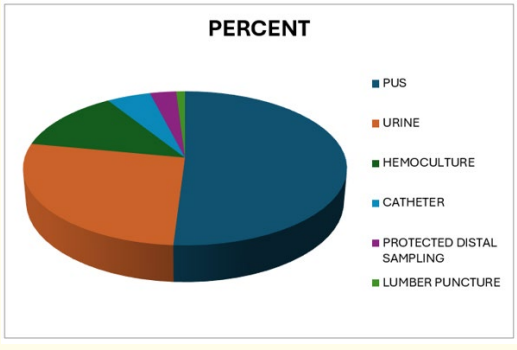

Figure 7: *Distribution of EPCs by type of sampling.*

EPC species isolated

In our series, we isolated six species of carbapenemase-producing enterobacteria from various samples, the most frequent being *Klebsiella pneumoniae* with a percentage of 54%, followed by *Escherichia coli* 33%, *Enterobacter cloacae* 6%, *Proteus mirabilis* 3% and *Citrobacter freundii* and *braakii* at 2% each (Figure 8).

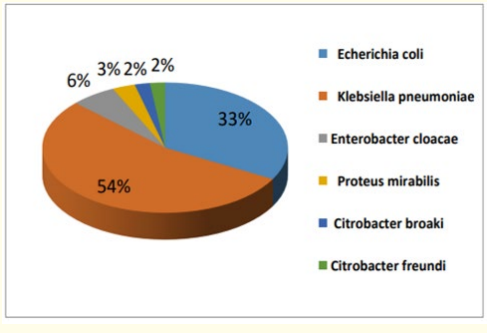

Figure 8: *Percentage of EPC species found.*

Carbapenemases among multi-resistant bacteria

The percentage of carbapenemases among multi-resistant bacteria (MRB) is 07%, ranking 3rd after ESBL: 49% and *Acinetobacter baumannii*: 43%. This percentage remains high compared with MRSA, which stands at 01% (Figure 9).

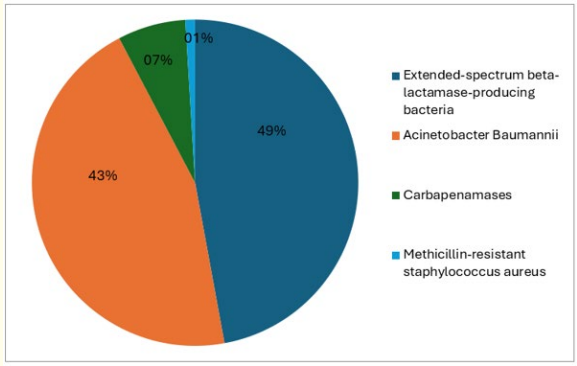

Figure 9: *Percentage of carbapenemases among multi-resistant bacteria (MRB).*

Types of carbapenemases diagnosed

In our series, we found two types of carbapenemases: OXA 48 and NDM, with the same percentage of 35.5%, i.e. a total of 39 patients. The remaining patients had a combination of both OXA 48 and NDM (Figure 10).

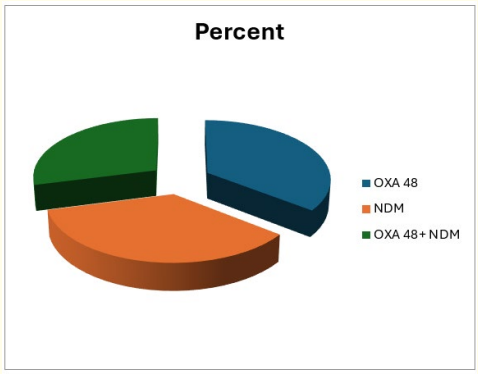

Figure 10: *Percentages of carbapenemase types found in our series.*

Antibiotic susceptibility profile

Of the 110 EPC strains isolated, 96% were resistant to sulphonamides and diaminopyrimidines (Bactrim), 94% to quinolones (norfloxacin, levofloxacin and ciprofloxacin) and 80% to aminoglycosides (Amikacin and gentamicin). On the other hand, 98% of isolates were sensitive to Colimycin (Colistin).

This profile was almost identical in all departments where EPCs were detected.

Evolution

The evolution of our patients after antibiotic treatment was marked by 35% cure and 32% death. The remainder survived with complications or sequelae.

We were unable to determine whether cases of death or disability were related to EPC infection or to the initial cause of hospitalization, but we did note that 66% of cases of death were secondary to a state of septic shock probably due to EPC infection.

Discussion

The most clinically and epidemiologically important carbapenemases are *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-betalactamase (NDM-1) and Oxacillinase (OXA-48). The first KPC was identified in 1996 in the United States, then spread to Israel, Italy, Greece, Romania, Colombia, Brazil, Argentina, China and Taiwan. The NDM-1 type was first isolated in 2009 in Sweden in a patient from India. A high prevalence of NDM-1 is currently also found in the Balkan region, northern Africa and the Arabian Peninsula. The carbapenemase OXA-48 was discovered in Turkey in 2003 and causes only a slight decrease in susceptibility to carbapenems, unless associated with other resistance mechanisms. Currently, the endemic countries for OXA-48 are Turkey, North Africa and India, and although there is a clear epidemiology for carbapenemase endemic countries, this can change as a result of factors such as migration, medical tourism and global trade. In addition, several countries not considered endemic have experienced recurrent epidemics of EPCs, which can be exported to neighboring countries [3].

This epidemiology is in line with some statistics in the literature, which show very high EPC rates in the USA, with a percentage of 64% according to a study by Thaden., *et al*. [6], and 74.50% in China [7]. In Africa, epidemiological data are variable, but always high in the Algerian region, with a percentage of 69.56% [8], in contrast to Morocco, where very low percentages can be distinguished, such as a study carried out in Rabat, with an EPC rate of around 6.05% [9], to which we can add our own study, with an EPC percentage of 1% among all enterobacteria and 7% among multi-resistant bacteria, which is very low compared with the studies previously cited. We can explain this finding by the fact that Morocco is one of the countries with recurrent epidemics.

The mean age at onset of EPC in our series was 49 years, which remains low compared with other countries such as France and Canada, where the mean ages are 70 and 69 respectively [10,11]. This can probably be explained by differences in living conditions, particularly in terms of hygiene, sanitation and immunity.

The occurrence of EPCs in pre-existing comorbidities is frequent in our series, particularly in immunocompromised settings, which is the case even in Tunisia, where the most dominant immunocompromising factor was diabetes, followed by France [11,12].

EPCs mainly occur in hospital settings, especially in wards with long hospital stays and heavy, multidisciplinary patient management, such as intensive care. However, we have noted a non-negligible rate of EPCs in intensive care units, with a percentage of 30%, which is also high in a study by Holman., *et al*. at 55.5%, and by Tidrarine., *et al*. at 41% [13,14].

The most common carbapenemase enterobacteria worldwide is *Klebsiella pneumoniae*. Several studies carried out in different parts of the world have shown that this is the most dominant EPC species, with 85.6% found in Spain, 65% in Switzerland and 65.7% in Belgium [15-17]. In Africa, results were similar, with *Klebsiella pneumoniae* being isolated in 85.2% of cases in Tunisia, and 84.8% in Rabat, Morocco [9,18]. In our study, this species was found in 54% of cases.

Most carbapenemase-producing *Enterobacteriaceae* are multiresistant to other antibiotic classes, which limits their therapeutic potential. This is often due to the association of a carbapenemase with an ESBL. In practical terms, the antibiotics of choice are colimycin, tigecycline, fosfomycin and a few quinolones. Tigecycline has been shown to be effective alone or in combination. A study of EPCs showed

that 70% of patients progressed well on tigecycline. The use of colimycin and aminoglycosides alone or in combination has had variable success. A study of carbapenemase-producing *Klebsiella pneumoniae* showed a 66.7% response to colimycin alone or in combination with aminoglycosides or tigecycline. A study by Akova M., *et al*. conducted on a series of carbapenemase-producing *Klebsiella pneumoniae* samples confirmed the value of combining colimycin with carbapenems, showing 80.5% success with this dual therapy, a high percentage compared with that of patients treated with monotherapy alone: 63.1% [19].

Mortality seems to be even lower when therapies combine two antibiotics to which the strain remains sensitive [20]. In our study, 94% of strains were resistant to quinolones (norfloxacin, levofloxacin and ciprofloxacin) and 80% to aminoglycosides (Amikacin and Gentamicin). However, 98% of isolates were sensitive to Colimycin (Colistin). All our patients were put on colimycin alone or in combination, with a response rate of 35%. On the other hand, we noted 32% cases of death, a percentage which is close to the results described in the literature, where death rates vary between 20 and 40%. In France, an EPC death rate of 35.7% was noted in a study by Bovin., *et al*. Another study conducted in France, again by Gordon., *et al*. and more recently, noted a rate of 23.1%. In the USA, Tamma., *et al*. reported an EPC death rate of 32% [21-23].

Among the measures to be taken to prevent the transmission of EPCs, it's worth remembering the importance of applying basic hygiene practices, rigorous asepsis, disinfection of common-use equipment, favoring single-use equipment, and using personal protective equipment. However, to cope with the growing threat of EPCs, basic practices alone are not enough, and other measures must be implemented, such as the choice and prescription of probabilistic antibiotic therapy, screening on admission or during hospitalization, and isolation of patients infected with BMRs in general and EPCs in particular.

Conclusion

This is an infection of considerable seriousness, and a shared responsibility involving the clinical physician and paramedical staff. Biologists play a crucial role in the rapid detection and management of this type of infection, not only in guiding antibiotic therapy, but also in monitoring the bacterial ecology of hospital wards.

Bibliography

1. Carmeli Y., *et al*. "Controlling the spread of carbapenemase-producing Gram-negatives: therapeutic approach and infection control". *Clinical Microbiology and Infection* 16.2 (2010): 102-111.

2. Nordmann P and Poirel L. "The difficult-to-control spread of carbapenemase producers among *Enterobacteriaceae* worldwide". *Clinical Microbiology and Infection* 20.9 (2014): 821-830.

3. Flaminia Olearo., *et al*. "Entérobactéries productrice de carbapénémases: stratégie de contrôle et prise en charge des patients porteurs 2017/05".

4. Martinez-Martinez L. "Extended-spectrum beta-lactamases and the permeability barrier". *Clinical Microbiology and Infection* 14.1 (2008): 82-89.

5. Levy SB and O'Brien TF. "Antimicrobial resistance alerts and implications". *Clinical Infectious Diseases* 41.4 (2005): S219-S220.

6. Thaden JT., *et al*. "Rising rates of carbapenem resistant *Enterobacteriaceae* in community hospitals: a mixed methods review of epidemiology and microbiology practices in a network of community hospitals in the southeastern United States". *Infection Control and Hospital Epidemiology* 35.8 (2014): 978-983.

7. Rui Z., *et al*. "Carbapenemase-Producing *Enterobacteriaceae* in Yunnan Province, China". *Japanese Journal of Infectious Diseases* 69.6 (2016): 528-530.

8. Larouci M., *et al*. "Prévalence des souches d'entérobactéries productrices de carbapénèmases isolées des infections communautaires et nosocomiales dans l'ouest algérien, Algerie 2017".

9. Akel Z and Zouhdi M. "Profil Épidémiologique Des Entérobactéries Productrices De Carbapénèmases Au Laboratoire De Microbiologie Du Chu Ibn Sina Rabat". Université Mohammed V (2014).

10. Fauvel M and Trembli C. "Rapport d'activités 2013-2014 du Laboratoire de santé publique du Québec". INSPQ (2015): 26.

11. Weber L., *et al*. "Traitement des infections dues aux entérobactéries productrices de carbapénèmases (EPC)". Congrès de la SFAR (2023): 6-7.

12. Manai T., *et al*. "Profil de résistance aux antibiotiques et caractéristiques moléculaires des souches productrices de carbapenemases". Le 30ème congrès national de la Société Tunisienne de Pathologie. Infectieuse.

13. Anne-Marie Holman. "Étude épidémiologique des entérobactéries productrices de carbapénèmase à La Réunion de 2010 à 2015". *Médecine Humaine et Pathologie* (2016).

14. Tidrarine S. "Epidémiologie des entérobactéries multirésistantes productrices de carbapénèmases à l'HIT" (2019): 101.

15. Jesús Oteo. "Carbapenemase-Producing *Enterobacteriaceae* in Spain in 2012". *Antimicrobial Agents and Chemotherapy* 57.12 (2013): 6344-6347.

16. Dortet L., *et al*. "Strategy for rapid detection of carbapenemase-producing *Enterobacteriaceae"*. *Antimicrobial Agents and Chemotherapy* 58.4 (2014): 2441-2445.

17. Shibl A., *et al*. "The emergence of OXA-48- and NDM-1 -positive *Klebsiella pneumoniae* in Riyadh, Saudi Arabia". *International Journal of Infectious Diseases* 17.12 (2013): 130-133.

18. Ben Helal., *et al*. "Occurrence and characterization of carbapenemase producing *Enterobacteriaceae* in a Tunisian Hospital". *Microbial Drug Resistance* 24.9 (2018): 1361-1367.

19. M Akova., *et al*. "Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria". *Clinical Infectious Diseases* 18.5 (2012): 439-448.

20. Nordmann A Carrer. "Les carbapénèmases des entérobactéries". Service de Bactériologie-Virologie-Hygiène, Hôpital de Bicêtre, 78, Rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France.

21. Bovin S. "Les entérobactéries productrices de carbapenemases". *Perspective Infirmière* 13.15 (2016): 53-56.

22. Godron A., *et al*. "Infections à entérobactéries productrices de carbapénèmases (EPC): Etude de cohorte retrospective". *Médecine et Maladies Infectieuses Formation* 1.2 (2022): 33-34.

23. D Tamma., *et al*. "Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* bacteremia". *Clinical Infectious Diseases* 64.3 (2017): 257-264.

Volume 7 Issue 8 August 2024