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REVIEW

Carbapenemase Producing Gram-Negative Bacteria in Tunisia: History of Thirteen Years of Challenge

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Abstract: The wide spread of multidrug-resistant bacteria, particularly carbapenem-resistant Gram-negative bacteria (CR-GNB), constitutes a major public health threat worldwide, owing to the limited therapeutic options. This review will describe and uncover the Tunisian experience in the challenge against carbapenem resistance. Indeed, we illuminate on the dissemination of CR-GNB in different hospitals, animals, and other natural environments in this country. We resumed the different carbapenemase variants detected from various bacterial species and mapped their regional distribution, basing on Tunisian published data during a period extended from 2006, the date of its first description in Tunisia, to February 2019. We also resumed the different mobile genetic elements implicated in their dissemination. This review shows that the majority of the research reports focused in the north and the coastal cities in spite of the fact that KPC and IMP carbapenemases were uncommonly detected in our country. However, VIM, NDM-1, and OXA-48 enzymes were usually reported with the predominance of OXA-48 among *Enterobacteriaceae*. Furthermore, OXA-23, OXA-51, and OXA-58 carbapenemases constituted the main mechanism conferring carbapenem resistance among *Acinetobacter baumannii* in Tunisia. Collaborative efforts and raising awareness of the threat of antibiotic resistance are required in order to minimize the spread of multidrug-resistant bacteria.

Keywords: Gram-negative bacteria, carbapenem resistance, carbapenemases, mobile genetic elements, Tunisia

Introduction

Global antibiotic consumption is steadily increasing, particularly in low-income countries, including Tunisia, compared to high-income countries that have managed to develop strategies for good control of antibiotic use.[1–3] Beta-lactams are the most used antibiotics in antibiotherapy. Accordingly, the importance of their use is justified by their therapeutic efficacy, their broad spectrum of action, and their low toxicity.[4,5] However, the use of antibiotics is not restricted to the human medicine but also in several sectors such as agriculture, animal husbandry, aquaculture, and water treatment.[6] Moreover, non-medical use of antibiotics contributed significantly to the global challenge of antibiotic resistance and the wide spread of multidrug-resistant bacteria. In fact, a potential risk to human health was described as the transmission of multidrug-resistant bacteria to healthy people through the food chain was observed.[7]

The crisis of antibiotic resistance was dramatically exacerbated by the global rise in consumption of the last resort of beta-lactam antibiotics, carbapenems which

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are considered as one of the treatments of choice against severe infections caused by multidrug-resistant bacteria.[8] Bacterial resistance to carbapenems was mainly conferred by carbapenemase production.[9] This mechanism was commonly described in Gram-negative bacteria. Indeed, more than 350 carbapenemase variants were currently identified (http://www.lahey.org/Studies/) and distributed in the entire world, noting the dominance of KPC, NDM-1, VIM, IMP, and OXA-48 enzyme types.[10] Besides, OXA-23 carbapenemase was frequently detected among carbapenem resistant *Acinetobacter baumannii*.[11]

In Tunisia, carbapenem consumption was folded during the period of 2011–2015. This is directly related to the increasing prevalence of severe infections caused by multidrug-resistant Gram-negative bacteria (GNB) (http://www.dpm.tn/). Several carbapenemases producing Gram-negative species have been recorded in Tunisia, as well as numerous carbapenemase variants which have been detected since 2006, the date of the emergence of the first carbapenemase producing isolate in Tunisia.[12]

In this review, we aimed to illuminate on the dissemination of different carbapenemase variants detected among CR-GNB isolated from various origins in Tunisia, based on Tunisian data published over 13 years from 2006 to 2019. This study enabled us to gain deep insight into the carbapenem resistance problem in Tunisia, in addition to mapping the regional distribution of carbapenemase enzymes, which is strongly encouraged by epidemiologists in order to improve surveillance strategies to minimize the spread of CR-GNB such as previously described.[13]

Carbapenemase Producing Gram-Negative Bacteria from Hospitals

Since 2006, the date of the first description of carbapenemase producing *Klebsiella pneumoniae* clinical isolate in a Tunisian hospital,[12] several types of carbapenemases have been detected in different species isolated from diverse clinical specimens and/or environmental hospitals, as shown in Table 1. KPC enzyme has recently been detected in *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates and is still uncommon in Tunisia.[14,15] This enzyme was firstly discovered in a clinical isolate of *Klebsiella pneumoniae* in North Carolina in 2001.[16] Since then, it has dramatically disseminated around the world, with a high prevalence in Greece, Italy, and Latin America,[17,18] whereas, it has rarely been detected in Africa.[19] The KPC enzyme was described in various members of *Enterobacteriaceae* and non-fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[18]

IMP metallo-β-lactamase type was scarcely reported in Tunisia, and it was observed only in *Klebsiella pneumoniae* isolated from the environment.[20,21] Several investigations revealed that Asian countries, especially Japan and China, were the main reservoirs of this enzyme.[9]

Moreover, we have noted that the VIM enzyme was the main metallo-β-lactamase detected in Tunisia, and was frequently reported among *Pseudomonas aeruginosa*, followed by *Klebsiella pneumoniae* (Tables 1 and 2). This enzyme was scarcely reported among *Escherichia coli* and *Enterobacter cloacae* in Tunisia.[20,22] In addition, the NDM-1 encoding gene was dramatically disseminated in Tunisia despite being lately reported comparing with other metallo-β-lactamases,[23,24] and it was mainly detected among *Klebsiella pneumoniae* (Tables 1 and 2). However, recent studies reported the detection of NDM-1 in *Acinetobacter baumannii* and *Proteus mirabilis*.[25,26]

Concerning class D carbapenemases, we have noted the circulation of four major subfamilies in Tunisia, namely; OXA-48-like, OXA-23-like, OXA-51-like, and OXA-58-like (Table 1). Besides, three OXA-48-like variants (OXA-48, OXA-204, and OXA-232) were found in *Enterobacteriaceae*, most commonly *Klebsiella pneumoniae*. OXA-48 production was considered as the main mechanism conferring resistance to carbapenems among *Enterobacteriaceae* in Tunisia, as well as Mediterranean countries where considered as endemic.[27] However, carbapenem resistance among *Acinetobacter baumannii* species was usually associated with the production of OXA-23, OXA-58, and the intrinsic OXA-51 carbapenemases, as shown in Table 1. OXA-51 encoding gene is intrinsic to all *Acinetobacter baumannii* isolates. Therefore, a high prevalence of this gene is expected when this species is studied. This gene will only lead to carbapenem resistance when it is adjacent to the insertion sequence IS*Aba1* which promotes its expression. Indeed, the simple detection of the gene does not imply that the strain is resistant to carbapenems.[28]

On the other hand, carbapenemase encoding genes were also detected in the hospital effluents. A recent study has recorded the presence of KPC enzyme in the effluent of three hospitals characterized by a higher patient volume and higher volume of carbapenem consumption. One of these hospitals is located in the capital city, whereas the two others are in coastal cities.[29] In contrast,

Table 1 Characteristics of Carbapenemase Producing Gram-Negative Bacteria in Tunisia from 2006 to 2019

Ambler Classes	Carbapenemases	Variants	Concerned Species	Number of Isolates (n)	Sequence Type	Origins	Locations	Isolation Years	Genetic Environment	References
A	**KPC**	KPC-2	*E. coli*	1	ST5700	Clinical	National Bone Marrow Transplant Center of Tunisia (Tunis)	2013	Transposon	[15]
		ND	*K. pneumoniae*	20	ND	Clinical	Rabta University Hospital of Tunis	2014	ND	[14]
		KPC-3	*E. coli*	1	ST167	Seafood (mussels)	Retail market	2015	Tn *4401d* Inc FII	[31]
		ND	ND	–	ND	Wastewater effluents from hospitals	Charles Nicolle Hospital (Tunis) Fattouma Bourguiba Hospital (Monastir) Farhat Hached hospital (Sousse)	2015–2016-2017	ND	[29]
B	**IMP**	IMP-1	*K. pneumoniae*	9	ND	Hospital environment	Kasserine Hospital	2010	ND	[20]
		IMP-8	*K. pneumoniae*	6	ND	Polluted-river	Oued Meliane (Rades)	2010	Class1 Integron	[21]
		IMP-10	*K. pneumoniae*	8						
		IMP-13	*K. pneumoniae*	2						
	VIM	VIM-4	*K. pneumoniae*	11	ND	Clinical	Habib Bourguiba University Hospital (Sfax)	2005	Plasmid Class1 Integron	[12]
		VIM-2	*P. aeruginosa*	30	ND	Clinical	Two hospitals in Sfax	2007	Chromosome	[53]
			P. aeruginosa	5	ND	Clinical	Sahloul University Hospital (Sousse)	2006–2007	Class 1 Integron Chromosome	[39]
			P. aeruginosa	35	ND	Clinical	Charles Nicolle Hospital (Tunis)	2002–2003–2004–2005–2006	Class 1 Integron	[38]
			P. aeruginosa	16	ND	Clinical	Charles Nicolle Hospital (Tunis)	2008	ND	[54]
			P. aeruginosa	9	ND	8 Clinical +1Hospital environment	Aziza Othmana Hospital (Tunis)	2007–2008	Class 1 Integron	[55]
			E. coli	4	ND	Hospital environment	Kasserine Hospital	2010	ND	[20]
		VIM-1	*K. pneumoniae* *H. pylori*	3 7	ND	Polluted-river	Oued Meliane (Rades)	2010	ND	[21]
		ND	*K. pneumoniae*	20	ND	Clinical	Rabta University Hospital of Tunis	2014	ND	[14]

(*Continued*)

Table 1 (Continued).

Ambler Classes	Carbapenemases	Variants	Concerned Species	Number of Isolates (n)	Sequence Type	Origins	Locations	Isolation Years	Genetic Environment	References
B	**VIM**	ND	*K. pneumoniae*	5	ND	Clinical	Military Hospital of Tunis	2014–2015–2016	ND	[56]
		VIM-2	*E. cloacae*	1	ND	Clinical	Regional Hospital of Djerba	2015–2016	Class1 Integron	[22]
	NDM	NDM-1	*K. pneumoniae*	1	ST11	Clinical	Military Hospital of Tunis	2012	Inc N	[23]
			K. pneumoniae	19	ST147	Clinical	Sahloul University Hospital (Sousse) Tahar Sfar hospital (Mahdia)	2013–2014–2015	Inc FIIk Tn*125*	[24]
			A. baumannii	11	ST85	Clinical	Sahloul University Hospital (Sousse)	2013–2014–2015	ΔTn*125* Chromosome	[25]
			K. pneumoniae	1	ST11	Clinical	Sahloul University Hospital (Sousse)	2015	Plasmid	[57]
			A. baumannii	1	ST85	Clinical	Fattouma Bourguiba Hospital (Monastir)	2013–2014–2015–2016	ΔTn*125* Chromosome	[40]
			K. pneumoniae	30	ST147 ST101	Clinical	Tahar Sfar hospital (Mahdia)	2015–2016	Inc FIIk Tn*125*	[43,58]
			K. pneumoniae	7	ND	Fecal and rectal carriage	Charles Nicolle Hospital (Tunis)	2014–2015	IS*Aba1*25	[47,59]
			K. pneumoniae	19	ST147 ST307	Clinical		2010–2011–2012–2013–2014–2015	Inc FIA IS*Aba1*25	[60]
			A. baumannii	2	ST1089	Fecal carriage		2014–2015	IS*Aba1*25	[61]
			K. pneumoniae	13	ND	Clinical	Military Hospital of Tunis	2014–2015–2016	ND	[56]
			K. pneumoniae	9	ST15 ST147 ST1412	Clinical	Regional Hospital of Djerba	2015–2016	Inc FIIA Inc A/C IS*Aba1*25	[22]
			P. mirabilis	1	ND	Clinical	Charles Nicolle Hospital (Tunis)	–	Plasmids	[26]
		ND	ND	-	ND	Wastewater effluents from 7 hospitals	Charles Nicolle Hospital (Tunis) Fattouma Bourguiba Hospital (Monastir) Farhat Hached hospital (Sousse) Tahar Sfar Hospital (Mahdia) Hedi Chaker Hospital (Sfax) Regional Hospital (Gafsa) Regional Hospital (Sidi Bouzid)	2015–2016–2017	ND	[29]

D	**OXA-48**	OXA-48	*K. pneumoniae*	21	ND	Clinical	Habib Bourguiba University Hospital (Sfax)	2009–2010	Inc A/C	[62]
			K. pneumoniae	2	ST101 ST383	Clinical	Private hospital in Tunis Military Hospital of Tunis	2010	Plasmid (60 kb) Tn *1999.1* Tn *1999.2*	[63]
			K. pneumoniae *C. freundii*	4 1	ND	Clinical	Charles Nicolle Hospital (Tunis)	2010–2011	Inc A/C (54 kb) Chromosome IS*1999*	[64]
			P. stuartii	13	ND	Clinical and Environmental	Habib Bourguiba University Hospital (Sfax)	2011	Inc A/C Tn *1999*	[65]
			K. pneumoniae	13	ND	Clinical	Military Hospital of Tunis	2011–2012	Inc A/C Inc R	[66]
			K. pneumoniae	1	ST11	Clinical	Military Hospital of Tunis	2012	Inc L/M	[23]
			K. pneumoniae	19	ST11 ST14 ST15 ST101 ST882 ST196 ST1978	Clinical	National Bone Marrow Transplant Center of Tunisia (Tunis)	2011–2012–2013–2014	ND	[15]
			E. coli	2	ST58 ST227					
			K. pneumoniae	4	ST15	Clinical	National Bone Marrow Transplant Center of Tunisia (Tunis)	2011	ND	[67]
			K. pneumoniae	5	ND	Clinical	National Bone Marrow Transplant Center of Tunisia (Tunis)	2013	ND	[68]
			K. pneumoniae	1	ST11	Clinical	The Maternity and Neonatology center of Monastir	–	Tn *1999.2*	[42]
			K. pneumoniae	1	ST147	Clinical	Tunisian Hospital	–	Inc L/M Tn *1999.2*	[69]
			K. pneumoniae	3	ST101	Clinical	Avicenne clinic (Tunis)	2014	ND	[70]
			K. pneumoniae *E. cloacae* *E. aerogenes**	2 1 1	ND	Rectal carriage	Charles Nicolle Hospital (Tunis)	2014	ND	[59]
			K. pneumoniae	13	ST101 ST147	Fecal carriage	Charles Nicolle Hospital (Tunis)	2014–2015	Tn *1999*	[47]
			K. pneumoniae	3	ST147	Clinical	Charles Nicolle Hospital (Tunis)	2010–2011–2012–2013–2014–2015	Plasmid Tn *1999*	[60]

(*Continued*)

Table 1 (Continued).

Ambler Classes	Carbapenemases	Variants	Concerned Species	Number of Isolates (n)	Sequence Type	Origins	Locations	Isolation Years	Genetic Environment	References
D	**OXA-48**	OXA-48	*K. pneumoniae*	1	ST147	Clinical	Sahloul University Hospital (Sousse)	2014	Inc L Tn *1999.2*	[24]
			K. pneumoniae	5	ST15 ST101	Clinical	Sahloul University Hospital (Sousse)	2012–2013–2014–2015–2016	Plasmid	[57]
			K. pneumoniae	1	ST101	Clinical	Tahar Sfar hospital (Mahdia)	2012	Inc L/M Tn*1999.2*	[71]
			K. pneumoniae	20	ST101 ST147 ST392	Clinical	Tahar Sfar hospital (Mahdia)	2015–2016	Inc L Tn *1999*	[43]
			K. pneumoniae *E. coli* *E. cloacae*	79 3 10	ND	Clinical	Military Hospital of Tunis	2014–2015–2016	ND	[56]
			K. pneumoniae	8	ND	Clinical	Military Hospital of Tunis	2015	ND	[46]
			K. pneumoniae *P. mirabilis*	5 1	ST15	Clinical	Regional Hospital of Djerba	2015–2016	NT Plasmid (48,5kb) Tn *1999.2*	[22]
			ND	-	ND	Wastewater effluents from 7 hospitals	Charles Nicolle Hospital (Tunis) Fattouma Bourguiba Hospital (Monastir) Farhat Hached hospital (Sousse) Tahar Sfar Hospital (Mahdia) Hedi Chaker Hospital (Sfax) Regional Hospital (Gafsa) Regional Hospital (Sidi Bouzid)	2015–2016–2017	ND	[29]
		OXA-204	*K. pneumoniae* *E. coli*	1 1	ST101 ST617	Clinical	The Maternity and Neonatology center of Monastir	–	Tn *2016*	[42]
			S. enterica	1	ST198	Clinical	Habib Bourguiba Hospital (Sfax)	2009	Inc A/C	[72]
			K. pneumoniae	1	ST147	Clinical	Sahloul University Hospital (Sousse)	2013	Plasmid	[57]
			K. pneumoniae	1	ST147	Clinical	Tahar Sfar hospital (Mahdia)	2013	Inc A/C IS*Ecp1*	[71]
			K. pneumoniae	4	ST147	Clinical	Tahar Sfar hospital (Mahdia)	2015–2016	Inc A/C Tn *2016*	[43]

D	**OXA-48**	OXA-204	*K. pneumoniae*	1	ND	Clinical	Military Hospital of Tunis	2011–2012	IncA/C	[66]
		OXA-232	*K. pneumoniae*	3	ST147	Clinical	Military Hospital of Tunis	2015	Plasmid	[46]
	OXA-23	OXA-23	*A. baumannii*	13	ND	Clinical	Sahloul University Hospital (Sousse)	2005–2006–2007	IS*Aba1* Chromosome	[52]
			A. baumannii	41	ND	Clinical	Charles Nicolle Hospital (Tunis)	2007	ND	[74]
			A. baumannii	2	ST1	Clinical	Military Hospital of Tunis Mohamed Kassab Orthopedic Institute (Tunis)	2006 2010	IS*Aba1* Chromosome	[75]
			A. baumannii	2	ND	Clinical	The Maternity and Neonatology center of Monastir	2011	Plasmid GR6 IS*Aba1* Chromosome	[44]
			A. baumannii	2	ST641	Clinical	Sahloul University Hospital (Sousse)	2012	Plasmid Gr6 Tn2008 IS*Aba1*	[76]
			A. baumannii	235	ST85 (ST1089)	Clinical	Sahloul University Hospital (Sousse)	2013–2014–2015	Plasmid (repAci6-family) Tn2008 IS*Aba1*	[25]
			A. baumannii	5	ND	Rectal carriage	Charles Nicolle Hospital (Tunis)	2014	ND	[59]
			A. baumannii	11	ST195	Fecal carriage		2014–2015	ND	[61]
			A. baumannii	25	ST2 ST1 ST164 ST310 ST570 ST602 ST623 ST636	Clinical	Mohamed Kassab Orthopedic Institute (Tunis)	2013–2014–2015–2016	ND	[73]
			A. baumannii	83	ND	Clinical	Fattouma Bourguiba Hospital (Monastir)	2013–2014–2015–2016	IS*Aba1*	[40]
			A. baumannii	3	ND	Clinical	Regional Hospital of Djerba	2015–2016	IS*Aba1* Chromosome	[22]
			A. baumannii	2	ST2	Seafood (mussel and Oyster)	Bizerte lagoon	2015–2016	ND	[32]

(*Continued*)

Table 1 (Continued).

Ambler Classes	Carbapenemases	Variants	Concerned Species	Number of Isolates (n)	Sequence Type	Origins	Locations	Isolation Years	Genetic Environment	References
D	**OXA-51**	OXA-51	*A. baumannii*	19	ND	Clinical	Sahloul University Hospital (Sousse)	2001–2002–2003–2004–2005	Chromosome	[45]
			A. baumannii	50	ND	Clinical	Charles Nicolle Hospital (Tunis)	2007	Chromosome	[74]
			A. baumannii	2	ST1	Clinical	Military Hospital of Tunis Mohamed Kassab Orthopedic Institute	2006 2010	Chromosome	[75]
			A. baumannii	5	ND	Rectal carriage	Charles Nicolle Hospital (Tunis)	2014	ND	[59]
			A. baumannii	13	ST195	Fecal carriage	Charles Nicolle Hospital (Tunis)	2014–2015	Chromosome	[61]
			A. baumannii	101	ND	Clinical	Fattouma Bourguiba Hospital (Monastir)	2013–2014–2015–2016	IS*Aba1* Chromosome	[40]
			A. baumannii	25	ST2 ST1 ST164 ST310 ST570 ST602 ST623 ST636	Clinical	Mohamed Kassab Orthopedic Institute (Tunis)	2013–2014–2015–2016	Chromosome	[73]
		OXA-66	*A. baumannii*	2	ND	Clinical	The Maternity and Neonatology center of Monastir	2011	Chromosome	[44]
		OXA-82	*A. baumannii*	2	ST641	Clinical	Sahloul University Hospital (Sousse)	2012	IS*Aba1* Chromosome	[76]
		OXA-94	*A. baumannii*	11	ST85 (ST1089)	Clinical	Sahloul University Hospital (Sousse)	2013–2014–2015	IS*Aba1* Chromosome	[25]
	OXA-58	OXA-58	*A. baumannii*	1	ST1	Clinical	Mohamed Kassab Orthopedic Institute (Tunis)	2013–2014–2015–2016	ND	[73]
		OXA-97	*A. baumannii*	19	ND	Clinical	Sahloul University Hospital (Sousse)	2001–2002–2003–2004–2005	Plasmid Chromosome IS*Aba3*	[45]

Note: **Enterobacter aerogenes* has been recently renamed *Klebsiella aerogenes*.[35]
Abbreviation: ND, not determined.

Table 2 The Frequencies of Carbapenemases Detected in Each Species in Tunisia from 2006 to 2019

	KPC		VIM		NDM-1		IMP		OXA-48		OXA-23		OXA-51		OXA-58		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
A. baumannii	–	–	–	–	14	12.28	–	–	–	–	424	100	230	100	20	100	688	55.62
P. aeruginosa	–	–	95	65.07	–	–	–	–	–	–	–	–	–	–	–	–	95	7.68
K. pneumoniae	20	90.91	39	26.71	99	86.84	25	100	222	86.72	–	–	–	–	–	–	405	32.74
E. coli	2	9.09	4	2.74	–	–	–	–	6	2.34	–	–	–	–	–	–	12	0.97
P. mirabilis	–	–	–	–	1	0.88	–	–	1	0.39	–	–	–	–	–	–	2	0.16
C. freundii	–	–	–	–	–	–	–	–	1	0.39	–	–	–	–	–	–	1	0.08
E. cloacae	–	–	1	0.69	–	–	–	–	11	4.30	–	–	–	–	–	–	12	0.97
E. aerogenes[*]	–	–	–	–	–	–	–	–	1	0.39	–	–	–	–	–	–	1	0.08
P. stuartii	–	–	–	–	–	–	–	–	13	5.08	–	–	–	–	–	–	13	1.05
S. enterica	–	–	–	–	–	–	–	–	1	0.39	–	–	–	–	–	–	1	0.08
H. pylori	–	–	7	4.79	–	–	–	–	–	–	–	–	–	–	–	–	7	0.57
Total	22	1.78	146	11.80	114	9.22	25	2.02	256	20.69	424	34.28	230	18.59	20	1.62	1237	100

Note: [*]*Enterobacter aerogenes* has been recently renamed *Klebsiella aerogenes*.[35]

NDM metallo-β-lactamase and OXA-48 class D enzyme were detected in the effluents of seven hospitals distributed throughout the country.[29]

In general, hospital effluents are discharged into the natural environment after treatment using wastewater treatment plants (WWTPs). As previously reported, The WWTPs are not effective for removing resistance genes, and microbial drug resistant bacteria, consequently, constitute a potential risk for the spread of carbapenemase producers in our natural ecosystems (waters, animals, soils, plants, …).[29,30]

Carbapenemase Producing Gram-Negative Bacteria from Other Origins

Detection of carbapenem-resistant bacteria in Tunisia was not limited to the hospitals, but also it was observed in natural sources (Table 1). Indeed, carbapenemase producing Gram-negative species were isolated from a polluted river, located in the north of the country, and exposed to urban, industrial and hospital effluents.[21] In this river, carbapenemase production was observed among *Klebsiella pneumoniae* isolates harboring metallo-β-lactamases encoding genes, namely $bla_{\text{IMP-8}}$, $bla_{\text{IMP-10}}$, $bla_{\text{IMP-13}}$, and $bla_{\text{VIM-1}}$. This latter was also detected among *Helicobacter pylori* species.[21] Moreover, a KPC-3 producing *Escherichia coli*, belonging to the ST167 clone, has been recently isolated from seafood bought from a Tunisian retail market.[31] Besides, another study reported the emergence of OXA-23 producing *Acinetobacter baumannii* ST2 in seafood collected from Bizerte lagoon that was contaminated by hospital effluents.[32] This wide spread of OXA-23 producing clone has been recently detected among two fish belonging to the *Pagellus acarne* species fished in the Mediterranean Sea of Algeria, near Bejaia, which is about 626.3 km away from Bizerte.[33] The emergence and spread of carbapenem-resistant Gram-negative bacteria in the aquatic environment in Tunisia, as well as all over the world, poses an alarming concern. This is certainly related to the pollution, which is caused mainly by the discharge of hospitals effluents into the natural and aquatic environment. Several studies have shown that wastewater treatment plants (WWTP) playa major role in the release of antibiotics and antibiotic-resistant bacteria into the environment, particularly in low-income countries where many wastewater treatments plants functions are ineffective.[34] Consequently, in order to preserve our

natural wealth, it was strongly advised to control hospital effluents that constitute the main reservoir of these multi-drug-resistant bacteria, especially carbapenemase producers.[29]

Regional Distribution of Carbapenemases

The emergence and rapid spread of carbapenem-resistant GNB in Tunisia encouraged scientific researchers and epidemiologists to investigate genes involved in resistance to carbapenems. As described in Figure 1A, the number of published articles per year was clearly increased, specifically after 2014, and indicated by the highest number recorded in 2018. This is closely related to the dissemination of CR-GNB throughout the country and the increased awareness of the importance of surveillance and control of multidrug-resistant bacteria in order to improve the health quality. A high prevalence of carbapenemase producers was detected in our country and they were mainly identified as *Acinetobacter baumannii*, followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Table 2). Besides, Figure 1B presents the number of published research papers per carbapenemase type and shows that the majority of the published data reported the detection of the OXA-48 enzyme. This latter is frequently found spread among *Klebsiella pneumoniae*, followed by *Providencia stuartii*, *Enterobacter cloacae*, and *Escherichia coli*. This enzyme was also detected in one isolate of each species of *Proteus mirabilis*, *Citrobacter freundii*, *Salmonella enterica*, and *Enterobacter aerogenes*, which was recently renamed *Klebsiella aerogenes*,[35] as shown in Table 2. However, the low number of published articles was recorded for those which reported the detection of IMP metallo-β-lactamase type and OXA-58 class D carbapenemases (Figure 1B).

We have mapped the regional distribution of the different carbapenemases detected in this country since its first description, but we have noticed that epidemiological studies and investigations of resistance mechanisms were not systematically studied either, certainly because of the absence of surveillance programs or even diagnostic microbiology laboratories with sophisticated assays in some regions. As presented in Figure 2, epidemiological studies published to date in Tunisia did not exemplify the situation in Tunisia and just focused on the north and the coastal cities. In addition, we have noted that KPC enzyme was detected only in the northern cities, in contrast to metallo-β-lactamases and class D carbapenemases, which were detected also in the south and center of the country.

Genetic Environment

Generally, carbapenemase encoding genes are carried by mobile genetic elements such as plasmids, insertion sequences, transposons, and integrons that are responsible for their rapid dissemination.[36] As shown in Table 1, several reports focused on studying the genetic environment of detected carbapenemase encoding genes. The bla_{KPC} gene was located in a conjugative plasmid belonging to the Inc FII group and inserted in the Tn*3*-like

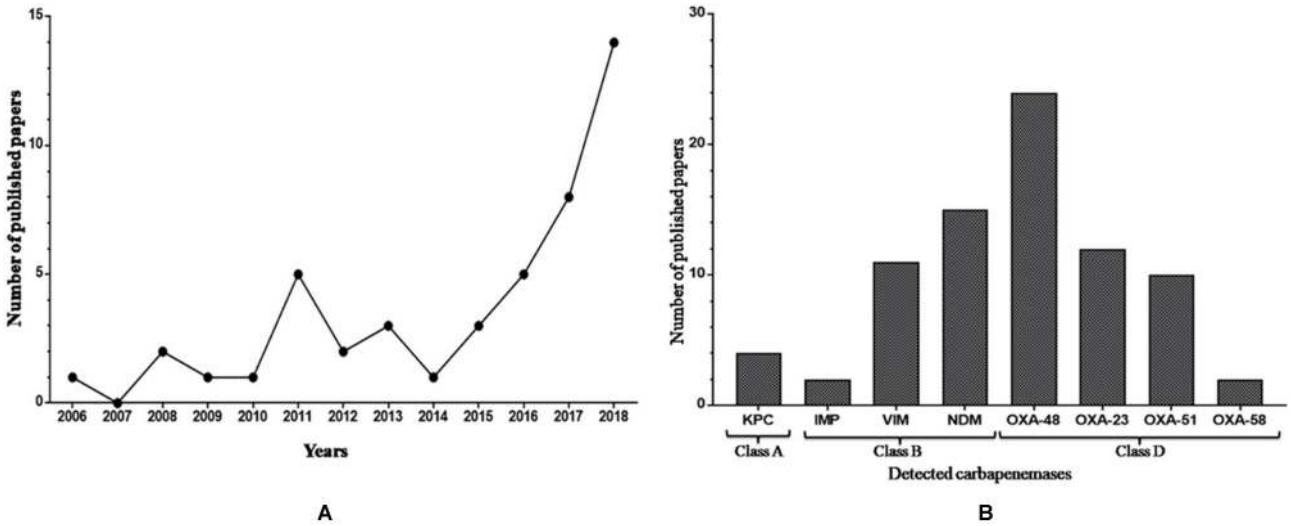

Figure 1 (**A**) Number of published papers reporting carbapenem-resistant Gram-negative bacteria in Tunisia from 2006 to 2019. (**B**) Number of published papers per carbapenemase classes in Tunisia from 2006 to 2019.

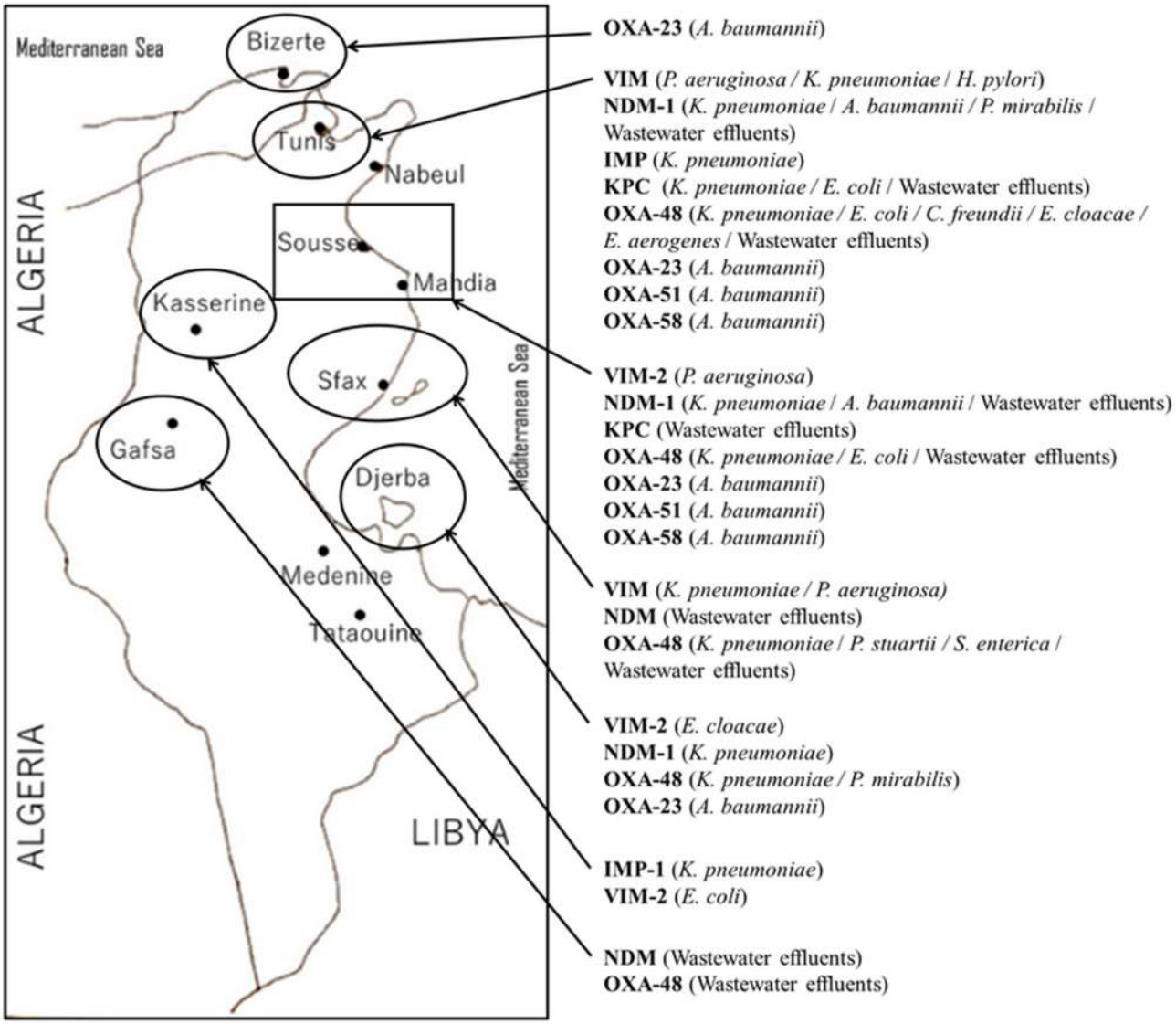

Figure 2 Regional distribution of carbapenemase producing Gram-negative bacteria in Tunisia from 2006 to 2019.

transposon, identified as Tn*4401d* and recognized as an active transposon enhancing the rapid spread of KPC encoding gene.[31,37] We cannot be certain that the bla_{KPC} gene is usually harbored by Inc FII plasmid because not all studies determined this. In fact, according to Table 1, only one *Escherichia coli* isolate was determined to carry the KPC encoding gene in this plasmid replicon type. Concerning class B metallo-β-lactamases encoding genes, it was demonstrated that the bla_{IMP} gene was inserted within the variable region of class 1 integrons.[21] This genetic structure was also found to be harboring the bla_{VIM} gene.[22,38,39] This latest was further found to be associated with the conjugative plasmids.[12] However, the bla_{NDM-1} gene was mainly found in plasmids which belonged to several incompatibility groups, such as Inc N, Inc FIIk, Inc FIA, Inc FIIA, and Inc A/C (Table 1). The characterization of the genetic structure surrounding the NDM-1 encoding gene showed that it was located in association with transposable elements, known as Tn*125*, flanked by two insertion sequences IS*Aba125* conferring a high potential of NDM-1 diffusion.[24,40,41]

Different plasmids were identified as harboring the bla_{OXA-48} gene; Inc A/C, Inc L/M, and Inc L, as described in Table 1. In addition, it was noted that the OXA-48 encoding gene was usually associated with the composite transposon Tn*1999.2*. In contrast, the $bla_{OXA-204}$ gene was inserted into the Tn*2016* transposon structure.[42,43] Besides, it was also revealed that the bla_{OXA-23} gene was

carried on the Tn*2008* transposon and associated with an upstream located insertion sequence IS*Aba1*. This gene was mainly detected on the chromosome, whereas few data described its plasmidic location.[25,44] According to the literature and as shown in Table 1, it was noted that the $bla_{\text{OXA-51-like}}$ gene, known as the intrinsic gene of *Acinetobacter baumannii*, was often detected on the chromosome backbone and sometimes linked to the insertion sequence IS*Aba1*.[40] OXA-58-like encoding gene was found to be carried either by chromosome or plasmids and associated with the insertion sequence IS*Aba3*.[45]

Sequence Types

As shown in Table 1, diverse sequence types (STs) were involved in the dissemination of carbapenemase encoding genes in Tunisia. Indeed, it was noted that KPC producing *Escherichia coli* isolates belonged to ST5700 and ST167, in contrast to those producing OXA-48-like which belonged to ST58, ST227, and ST617 proving the non-clonal dissemination of carbapenemase producing *Escherichia coli* in Tunisia.[15,31,42] Several sequence types of NDM-1 producing *Klebsiella pneumoniae* were recorded, including ST11, ST15, ST147, ST101, ST307, and ST1412, noting the predominance of ST147.[22–24,46] This latest was also described as the main producer of OXA-48 enzyme in this area, despite the detection of other sequence types such as ST11, ST15, ST14, ST101, ST383, and ST392 (Table 1). Only one OXA-204 producing *S. enterica* isolate, which belonged to ST198, was hitherto reported.

Regarding non-fermentative GNB, NDM-1 dissemination is associated to only one sequence type *Acinetobacter baumannii*, which designed ST85 according to the Pasteur database and ST1089 according to Oxford schema.[25,40,47] This sequence type was also detected as a producer of OXA-23 carbapenemase type.[25] In addition, several sequence types of carbapenemase class D enzyme producing *Acinetobacter baumannii* were further detected, including ST1, ST2, ST195, ST641, ST164, ST310, ST570, ST602, ST623, and ST636, noting the dominance of ST2 (Table 1).

Treatments

Infections caused by carbapenemase producing Gram negative bacteria, usually qualified as multidrug resistant, constitute a real concern. Indeed, these bacteria are characterized by a high frequency of co-resistance leading to the restriction of treatment choices and presenting real challenges in therapeutic decision-making.[48] Few antibiotics were destined for the treatment of these severe infections; polymyxin B (colimycine), polymyxin E (colistin), tigecycline, fosfomycin and some selected aminoglycosides that could be used in combination with carbapenems.[49] The clinical treatment of some carbapenemase producers seems to be extremely difficult, quoting the example of *P. mirabilis* which is intrinsically resistant to colistin and tigecycline;[26,50] emergence of such isolates constitutes a real threat.

Several studies addressed the effectiveness of the chosen therapy,[49] but herein the Tunisian experience in this field will be reviewed. Regardless of the treatment failure of some cases that ended in patient death, it was very interesting to note the treatment success of several patients. This is closely related to the infection sites and patient response with medication. Indeed, a successful treatment of carbapenemase producing *Klebsiella pneumoniae*, causing bloodstream infections and co-harboring $bla_{\text{VIM-2}}$, $bla_{\text{OXA-48}}$, bla_{SHV}, and $bla_{\text{CMY-2}}$, with high dose combination of two antibiotics (Imipenem + Amikacin) was recorded.[51] This success was also observed when treating patients, suffering from urinary infections caused by VIM-4 producing *Klebsiella pneumoniae*, with a combination of Imipenem and colimycine that was not effective for those suffering from bacteremia.[12] Moreover, it was reported that successive treatments with tigecycline/amikacin and fosfomycin of carbapenemase producing *Klebsiella pneumoniae*, showing colistin resistance, was also required.[43] Another study reported the successful treatment of NDM-1 producing *Klebsiella pneumoniae* with a combination of (fosfomycin/amikacin), (imipenem/fosfomycin/colistin), or (cefotaxime/tigecycline/imipenem).[24] Moreover, a successful treatment of OXA-23 producing *Acinetobacter baumannii* infection was observed using an association of ceftazidime with amikacin or gentamicin.[52]

Conclusion

The spread of multidrug-resistant Gram-negative bacteria throughout Tunisia, especially carbapenemase producers, constitutes a real concern. Indeed, we have noted the dissemination of several carbapenemase types among various Gram-negative species, showing a geographical variation that could be explained by the variability of hospital hygiene measures, epidemiological factors, and antibiotic use policies between different Tunisian healthcare settings. Our review is limited to the published data since

the first description of carbapenemase production in Tunisia to February 2019, and we should also note the absence of reporting in several regional hospitals because of the absence of surveillance programs or even diagnostic microbiology laboratories with sophisticated assays. Furthermore, the present study reveals the absence of published Tunisian data reporting the carbapenems consumption in the veterinary field as well as the detection of carbapenemases producing Gram-negative bacteria among breeding animals and farms in our country. The lack of such data in this field could be explained by the use of other antibiotics in farms, quoting the example of colistin. This latter has been considered as a successful solution for treatment of carbapenem resistant bacterial infections. However, the spread of colistin resistant encoding genes among breeding animals, patients, and community could exacerbate the crisis, conducting to the pandrug resistance. Intensifying efforts of clinicians, scientists, veterinaries, and ecologists are very required in order to curb the antibiotic resistance challenge which is considered one of the top priorities to improve the health system and to preserve the natural wealth in Tunisia.

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Author Contributions

All authors contributed to the data analysis, drafting, or revising of the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

The author Olfa DZIRI designed the study and was interested in extracting all Tunisian published data, processing them, and drafting the text, tables, and figures.

The author Raoudha DZIRI revised the draft paper and helped in the preparation of the study's analytical strategy.

The two authors, Allaaeddin Ali EL SALABI and Chedly CHOUCHANI revised the paper, ensuring the quality and the scientific information of the article.

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