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Emergence of NDM in Association with OXA-48 Carbapenemase in Raoultella Terrigena from a Moroccan University Hospital in Casablanca, Morocco

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ABSTRACT

Objective: The purpose of this study was to investigate the emergence of *Raoultella terrigena*, a rarely found opportunistic pathogen, co-producing NDM and OXA-48 carbapenemases in isolates recovered from various clinical specimens in our institution.

Methods: From April 2020 to November 2021, we focused on strains of *Enterobacteriaceae* with reduced sensitivity to ertapenem giving rise to suspicion of the production of carbapenemases according to the CASFM-EUCAST criteria. These strains were isolated from samples for diagnostic purposes and identified in the microbiology laboratory of our institution. Only non-duplicate clinical and surveillance isolates obtained from patients were included.

Results: Out of 159 Enterobacteriaceae suspected, 53 strains of *Raoultella terrigena* producing carbapenemases were isolated. Among these isolates, *64%(n=34)* were NDM producers, *4% (n=2)* were OXA-48 producers and *32% (n=17)* had both the NDM and OXA-48. The co-existence of NDM and OXA-48 carbapenemases was found more often in *Raoultella terrigena* than *Klebsiella pneumoniae*. Among the seventeen isolates of *Raoultella terrigena*, all were multidrug-resistant.

Conclusion: This study reported for the first time the emergence of NDM and OXA-48 carbapenemases co-existence in *Raoultella terrigena* isolates in our country which is starting to limit treatment options.

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KEYWORDS:
Raoultella terrigena, Morocco, carbapenemases, NDM, OXA-48.

INTRODUCTION

Raoultella terrigena is among the most uncommon nosocomial pathogens, causing serious infections with high mortality.(1) Indeed, *Raoultella terrigena* is a rarely found opportunistic pathogen that can cause healthcare-associated infections. However, in the Ibn Rochd university hospital center of Casablanca it is one of the most frequently isolated pathogens in the microbiology laboratory in recent years which makes our context interesting. Moreover, we notice lately the emergence of multi-resistant strains and more particularly the emergence of an NDM in association with OXA-48 carbapenemase producing *Raoultella terrigena*. Currently, carbapenems are among the few last-line drugs available for therapy against serious infections, such as pyelonephritis and bacteraemia, caused by multidrug-resistant *Enterobacteriaceae* species such as *Raoultella terrigena*.

The emergence of these carbapenemase-producing *Enterobacteriaceae* is a real public health problem and knowledge of their epidemiology is essential to control their spread.(2)

In this study, we report, for the first time, the emergence of NDM carbapenemase in association with OXA-48 in *Raoultella terrigena* isolated at the bacteriology laboratory of the University Hospital Ibn Rochd-Casablanca.

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MATERIALS AND METHODS

Setting

This study was conducted at the Ibn Rochd university hospital center, located in Casablanca city, a 1600 bed teaching hospital with three major branches; Ibn Rochd hospital, Al harouchi pediatric hospital, and 20 Aout 1953 hospital.

Study Design

This is a longitudinal cross-sectional study carried out in the bacteriology-virology and hospital hygiene laboratory of the Ibn Rochd-Casablanca University Hospital over a period of twenty months from april 2020 to november 2021.

Data collection of bacterial strains

It included Enterobacteriaceae isolated from samples for diagnostic purposes and focused on strains with reduced sensitivity to ertapenem giving rise to suspicion of the production of carbapenemases according to the CASFM-EUCAST criteria. Duplicates were excluded.

Antimicrobial identification and susceptibility testing

The identification of the bacterial species was carried out according to standard microbiological criteria using API 20 system *(biomérieux, Marcy-l'Etoile, France)* and the quality control strain ATCC *E.coli* 25922 was used. Antimicrobial drug susceptibility was determined by the disk diffusion method on Mueller–Hinton (MH) agar plates *(Bio-Rad,* Marnes-la-Coquette, France*)* according to the recommendations of CASFM-EUCAST 2020/2021.

Screening for the carbapenemase production: The CASFM-EUCAST Algorithm V1.2 2020

All carbapenem-resistant strains suspected of producing carbapenemases were subjected to the CASFM-EUCAST algorithm for EPC (Enterobacteria Producing Carbapenemase) screening with some modifications (ticarcillin/clavulanic acid, not tested for unavailable records).(3)

Detection and confirmation of carbapenemase

The presence of carbapenemases was confirmed by the rapid immunochromatographic test for the detection and identification of carbapenemases.: RESIST-5 O.O.K.N.V. K-Set (CORIS Bioconcept, Belgium).(4)

RESULTS

During this period, 159 non-duplicated strains of *Enterobacteriaceae* suspected of producing carbapenemases were included and 87% (138 strains) were carbapenemases producing *Enterobacteriacae*.

The presence of a carbapenemase was mainly found in: *Klebsiella pneumoniae 39,1%*(n=54), *Raoultella terrigena 38,4%* (n=53), *Escherichia coli* 13% (n=18) and 9,4% (n=13) in others (*Citrobacter koseri, Proteus mirabilis, Klebsiella oxytoca, Enterobacter cloacae, Serratia marcescens and Pantoea*).

Among the 53 strains of *Raoultella terrigena* producing carbapenemases, 34 strains (64%) were NDM producers, 2 strains (4%) were OXA-48 producers and 17strains (32%) had both the NDM and OXA-48.

Comparaison between *Raoultella terrigena* and *Klebsiella pneumoniae*

In comparison with *Raoultella terrigena*, among the 54 strains of *Klebsiella pneumoniae* producing carbapenemases, 39 strains (72%) were NDM producers, 10 strains (19%) were OXA-48 producers and 5 strains (9%) had both the NDM and OXA-48.

The co-existence of NDM and OXA-48 carbapenemases was found more often in *Raoultella terrigena* (32%) than *Klebsiella pneumoniae* (9%).

Co-existence of NDM and OXA-48 in carbapenem resistant *Raoultalla terrigena* isolates

These 17 strains of *Raoultella terrigena* were distributed as follows.

The age of these patients ranged from 8 to 79 years and the sex ratio was 1,1.

Figure 1 shows the distribution of strains according to the sample type: blood cultures were predominant with 59%, followed by urine with 23%, central catheters with 12% and peritoneal fluids with 6%.

The distribution of strains according to departments was as follows: the isolates came mainly from surgical wards (n=7), hematology (n=4), and intensive care units (n=4).

Among these 17 strains, all were resistant to antimicrobial agents tested in our laboratory: ampicillin, amoxicillin/ clavulanic acid, cephalothin, cefoxitin, cefotaxime, ceftazidime, aztreonam, imipenem, ertapenem, ciprofloxacin, gentamicin, tobramycin, netilmicin, amikacin and trimethoprim/sulfamethoxazole. Moreover, 15 strains expressed a confirmed expended spectrum b-lactamase (ESBL) while 2 strains were not tested for production of ESBL. *(Table1)*

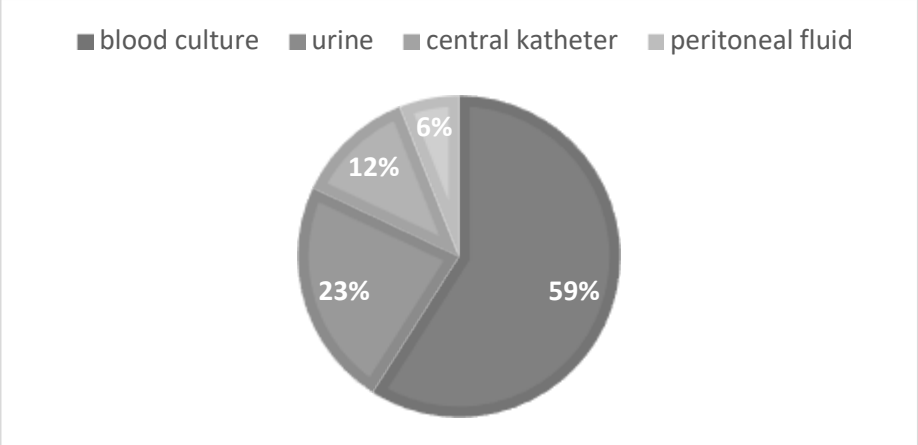

Figure 1. Distribution of the 17 isolates of *Raoultella terrigena* according to the nature of the sample

Table1. Caracteristics of the 17 isolates of *Raoultel*

Isolate No.	Date of isolation (day/month/year)	Age of patient	Gender	Department	Site of isolation	Production of ESBL
1	14/04/2020	40	M	Hematology	Central catheter	Not tested
2	14/05/2020	78	F	ICU	Blood culture	Confirmed
3	26/05/2020	79	M	ICU	Blood culture	Confirmed
4	08/12/2020	59	M	ICU	Blood culture	Not tested
5	04/03/2021	35	F	Surgical ward	Central katheter	Confirmed
6	15/03/2021	18	F	Surgical ward	Blood culture	Confirmed
7	15/03/2021	8	F	Surgical ward	Blood culture	Confirmed
8	16/03/2021	42	M	Infectious diseases	Blood culture	Confirmed
9	14/04/2021	69	F	Surgical ward	Urine	Confirmed
10	28/05/2021	42	M	ICU	Blood culture	Confirmed
11	01/06/2021	68	F	Surgical ward	Peritoneal fluid	Confirmed
12	10/06/2021	65	M	Nephrology	Urine	Confirmed
13	14/06/2021	42	F	Surgical ward	Urine	Confirmed
14	21/06/2021	40	F	Hematology	Blood culture	Confirmed
15	12/07/2021	44	M	Hematology	Blood culture	Confirmed
16	03/08/2021	51	M	Hematology	Blood culture	Confirmed
17	01/11/2021	69	M	Surgical ward	Urine	Confirmed

DISCUSSION

Raoultella terrigena is a gram-negative, capsule-forming bacillus belonging to the *Enterobacteriaceae* family. They are facultative anaerobes with both respiratory and enzymatic metabolism. *Raoultella terrigena* is an opportunistic pathogen isolated very rarely in the world and causing healthcare-associated infections with a high mortality (up to 44%). Indeed, infection by *Raoultella terrigena* can cause damage to various organs, especially in patients with chronic diseases. The origin of those infections can be endogenous (faeces and bile) and exogenous (water, milk and soil). Until now, it is unclear whether *Raoultella terrigena* is part of the normal human gut microbiota or an asymptomatic carrier of the pathogen. *Raoultella* species are normally found in aquatic environments, fresh water, plants and soil. (1)

It is really difficult to establish a specific clinical symptomatic profil for this pathogen or a clear pattern of disease progression because there are just few cases of *Raoultella terrigena* infection described separately and in detail in the world. However, the variety of clinical manifestations do not differ from those characteristics of representatives of the *Enterobacteriaceae* family or the *Klebsiella* genus.

The actual level of infection by *R. terrigena* may be higher because of the difficulty in distinguishing between complications of *R. terrigena* and *Klebsiella*. In addition, it should be noted that the genus *Raoultella* was recently separated from *Klebsiella* in 2001 based on analysis of 16S rRNA sequences and the rpoβ gene.(5)

The role of *Raoultella terrigena* in the pathogenesis of disease is underestimated and the identification and study of cases of infection with this bacterium is a promising and important area of modern clinical microbiology.(6) The introduction of accurate techniques in microbiology laboratories has led to an increase in the number of reports of infections with *Raoultella* species strains.

Cases of infection with carbapenem-resistant *Raoultella spp* have been sporadically reported. In recent years, despite the high mortality in clinical infections, there has not yet been a systematic study of the mechanisms of carbapenem resistance in *Raoultella* strains and difficulties in the correct identification of the pathogen still arise, which lead to an underestimation of its presence.(7)

Universal natural resistance to ampicillin is observed for all *Raoultella* isolates since the genome of representatives of this genus contains the class A β-lactamase gene. (8) In general, these bacteria are susceptible to amoxicillin, piperacillin, piperacillin-tazobactam, 2nd, 3rd, and 4th generation cephalosporins, carbapenems, aminoglycosides, trimethoprim/sulfamethoxazole, and tigecycline. Resistance genes to these drugs are located in bacterial plasmids and are not part of their genome.(1)

Carbapenemase-producing strains of *Enterobacteriaceae* have a very high epidemic potential. There emergence requires rapid identification of infected and carrier patients and it is essential to have effective diagnostic techniques. The resist-5 O.O.K.N.V. immunochromatographic test for the detection and identification of carbapenemases allows the rapid detection of one or more carbapenemases (OXA-48, NDM, VIM, KPC, and OXA-163) directly from a bacterial colony growing on any culture medium.
Moreover, this test allows the detection of OXA-48 and NDM carbapenemases even simultaneously with a sensitivity and specificity of 100% and a concordance of 100% with PCR. (4,9)

The genes coding for carbapenemases are most often located on plasmids that can be transferred from one strain to another, but also between 2 closely related species. It is therefore essential to identify strains harboring this type of transferable mechanism because of the potential danger they represent as sources, reservoirs, and vehicles of carbapenemase genes. It is possible to observe in some patients strains belonging to different species of *Enterobacteria* and producing the same carbapenemase, probably due to plasmid transfer between the two species in the host's gastrointestinal tract. (2,10,11)

The best known carbapenemases are represented by three molecular classes of b-lactamases: Ambler class A (KPC types), B (VIM and NDM enzymes) and D (OXA-48).(12) These enzymes confer high-level resistance to most b-lactam antibiotics such as penicillins and cephalosporins, but variably affect carbapenems. Nowadays, unfortunately, the widespread use of carbapenems has led to the emergence of resistant *Enterobacteria* in various regions of the world, including *Raoultella terrigena*, which is increasingly prevalent in our moroccan hospital. Of the seven strains isolated from the surgical wards, four were from the burn surgery and reconstruction department. This could be explained by the circulation of a clone in the burn surgery department during the study period.This constitutes nowadays a major therapeutic and epidemiologic challenge.

The New Delhi metallo--lactamase-1 (NDM1) carbapenemase, initially identified in *Escherichia coli* and *Klebsiella pneumoniae*, has rapidly spread in the world.(13) However, the combination of NDM carbapenemases in association with OXA-48 has never been reported in *Raoultella Terrigena* besides our Ibn Rochd university hospital center of Casablanca.(4)

This combination of NDM-1 carbapenemases in association with OXA-48 has already been described in several *Enterobacteria*, notably *Klebsiella pneumoniae, Escherichia coli, Serratia marcescens and Enterobacter cloacae*. (14) Concerning *Klebsiella pneumoniae*, we find this association of carbapenemases in Tunisia (15), Egypt (16), Pakistan (17), India (18), Switzerland(19), Italy(20)...

All these studies highlight the successful dissemination of carbapenem resistance genes in high-risk clones and emphasize the importance of detecting the spread of carbapenemases in other species such as *Raoultella terrigena*.

CONCLUSION

In Morocco, the emergence and rapid spread of carbapenemase-producing *Enterobacteriaceae* is alarming. We report for the first time the association of carbapenemases OXA-48 and NDM-1 in multidrug-resistant *Raoultella terrigena* from samples in a Moroccan university hospital. The spread of these isolates is starting to limit treatment options. Therefore, rapid treatment of patients carrying these strains is necessary to control and minimize the possibility of new reservoir formation. *R. terrigena* is a potential emerging pathogen and its incidence and pathogenic role are not well reported. Microbiologists and clinicians must now be aware of this threat and implement the necessary control measures to prevent possible spread in the population. The country's

health care institutions must be aware of the emergence of these multidrug-resistant strains, as they constitute a significant public health problem and strengthened health surveillance process must be implemented.

Table1. Caracteristics of the 17 isolates of *Raoultella terrigena*

Isolate No.	Date of isolation (day/month/year)	Age of patient	Gender	Department	Site of isolation	Production of ESBL
1	14/04/2020	40	M	Hematology	Central catheter	Not tested
2	14/05/2020	78	F	ICU	Blood culture	Confirmed
3	26/05/2020	79	M	ICU	Blood culture	Confirmed
4	08/12/2020	59	M	ICU	Blood culture	Not tested
5	04/03/2021	35	F	Surgical ward	Central katheter	Confirmed
6	15/03/2021	18	F	Surgical ward	Blood culture	Confirmed
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8	16/03/2021	42	M	Infectious diseases	Blood culture	Confirmed
9	14/04/2021	69	F	Surgical ward	Urine	Confirmed
10	28/05/2021	42	M	ICU	Blood culture	Confirmed
11	01/06/2021	68	F	Surgical ward	Peritoneal fluid	Confirmed
12	10/06/2021	65	M	Nephrology	Urine	Confirmed
13	14/06/2021	42	F	Surgical ward	Urine	Confirmed
14	21/06/2021	40	F	Hematology	Blood culture	Confirmed
15	12/07/2021	44	M	Hematology	Blood culture	Confirmed
16	03/08/2021	51	M	Hematology	Blood culture	Confirmed
17	01/11/2021	69	M	Surgical ward	Urine	Confirmed

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