


# Emergence of OXA-48 Carbapenemase Producing *Klebsiella pneumoniae* in a Neonatal Intensive Care Unit in Marrakech, Morocco

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## ABSTRACT

**GOAL:** This work aims to describe and explore the circumstances of appearance of *Klebsiella pneumoniae* producing OXA-48 carbapenemase, which has occurred in a neonatal intensive care service at the Mohammed VI University Hospital of Marrakech.

**RESULTS:** During February 2015, the alert was triggered by the isolation of 6 isolates of *K pneumoniae* with the same antibiotic susceptibility profile in the neonatal intensive care service, suggesting a possible outbreak. Blood cultures represented the main site of isolation of these isolates. The phenotypic study of the isolates made it possible to identify a strain of *K pneumoniae* susceptible to third-generation cephalosporins, ciprofloxacin, and aminoglycosides, and resistant to ertapenem,  $\beta$ -lactamases inhibitors (ticarcillin-clavulanate, piperacillin-tazobactam; amoxicillin-clavulanic acid), and cotrimoxazole. The genotypic study of the epidemic isolate revealed the presence of the *bla*<sub>OXA-48</sub> gene. The action to be taken was the establishment of corrective measures to stop this epidemic to a multi-resistant germ transmitted by hand transmission. The reinforcement of hygiene measures and the awareness of the staff made it possible to put an end to the epidemic at March 30, 2015, without closing the service. The outcome of 6 infected newborns was fatal due to the fragile terrain and the inappropriate probabilistic antibiotic therapy.

**CONCLUSION:** The production of carbapenemase in *K pneumoniae* is an emerging resistance mechanism that must be suspected and identified to offer targeted therapy and to limit its spread. The implementation of a local policy to control multidrug-resistant germs is essential to limit their dissemination in hospitals.

**KEYWORDS:** *K pneumoniae*, OXA-48, resistance, epidemic

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## Introduction

In Morocco, the appearance of resistance of the carbapenems in enterobacteriaceae by producing carbapenems OXA-48 has been proclaimed to an increasing extent and represented currently as a major matter of clinical concern, especially in resuscitation case. As a matter of fact, the producer strains of the carbapenemase are often multi-resistant because they merge multiple mechanisms of resistance which can reach a therapeutic impasse.

OXA-48, carbapenemase of class D, is one of the most widespread carbapenemase in the Mediterranean area.<sup>1,2</sup> This carbapenemase is encoded by the *bla*<sub>OXA-48</sub> gene. The gene is mainly detected as part of the composite transposon Tn 1999 in 2 copies of the insertion sequence IS1999.<sup>3</sup> Nonetheless, the resistance to cephalosporin of third generation and extended-spectrum  $\beta$ -lactamases (ESBLs) are frequently present in OXA-48 producers.<sup>1</sup>

The OXA-48 enzyme remains the most found carbapenemase in enterobacteria of diminished susceptibility to

carbapenems in Tunisia; this enzyme presents a diffusion in the Mediterranean basin (Lebanon, Israel, Egypt, Algeria, and France).<sup>4,5</sup>

The objective of this study is to describe and scout about the circumstances of the occurrence of an epidemic due to carbapenemase producing *K pneumoniae* which has been taking place in a neonatal resuscitation unit at Mohammed VI University Hospital in Marrakech.

## Patients and Methods

### Description of the setting

The hospital of mother and child has a capacity of 247 beds, with 2 services in pediatrics, neonatology, pediatric resuscitation, visceral and traumatic pediatric surgery, and pediatric emergencies.

At the emergency service, there is no incubator; there are just adult beds for mothers and their newborns.

The neonatology service is divided into 9 boxes and some of them contain 2 incubators.



### Chronological description of the epidemic

On February 25, 2015, a newborn hospitalized in neonatal resuscitation unit was identified as a carrier of catheter-related infection to a *Klebsiella pneumoniae* resistant strain to ertapenem and  $\beta$ -lactamases inhibitors. It was a newly born transferred from a provincial hospital for support of the neonatal respiratory distress on prematurity.

Within 5 following days of this first case, 2 cases of bacteremia caused by a strain with the same phenotypic profile were recorded. One week later, 3 new cases of bacteremia and urinary tract infections have been identified in the same department, caused by the same strain of *K pneumoniae* having the same phenotypic profile on the antibiogram (Table 1).

In total, 6 children were infected after the first index case, 3 of whom are premature. The average age of these children who have been infected with the strain was 2 days, and the sex ratio was 2. The entire newly borns infected with the same strain have been passed through pediatric emergencies. The hospitalization's average duration was 6 days. Blood strain was the main isolation site of these strains of *K pneumoniae*. The positivity time varied from 7 days to 1 day for the index case which remained in pediatric emergencies 48 hours before its shift to neonatology for the support of neonatal respiratory distress on prematurity.

The time limit between hospitalization in neonatal resuscitation and the diagnosis of infection differed from 4 to 7 days. Yet, a strain of *K pneumoniae* with the same phenotypic profile was identified at the level of pediatric emergencies from a cyto-bacteriological test of urine among a newly born child before his hospitalization in neonatal resuscitation unit.

All children received a probabilistic antibiotic therapy with ceftriaxone and gentamicin, which was replaced 48 hours later by non-clinical improvement of the patients by the combination of imipenem and amikacin in 2 cases.

During the course of this epidemic, the evolution was fatal with the death of all the newborns infected by this strain in the context of a severe sepsis in spite of the sensitivity of the strain in vitro to the antibiotics administered for some in probabilistic antibiotherapy.

### Investigations

The bacterial identification of all isolates was made according to conventional methods, and the study of antibiotic susceptibility was performed and interpreted according to the standards of the French Society of Microbiology (CASFM).

The bacterial identification was made by the Api 20 E (BioMérieux®) galleries and by the Phoenix BD automaton. The antibiogram was carried out according to the recommendations of CASFM EUCAST by diffusion method on agar medium and by determination of the minimal inhibitory concentration (MIC) on liquid medium on the automaton BD.

**Table 1.** Clinical, evolutionary, and therapeutic data of the cases of this epidemic.

CASE	AGE, DAYS	SEX	DIAGNOSTIC	SITE	POSITIVITY TIME LIMIT	ATB FIRST INTENTION	DURATION, D	ATB SECOND INTENTION	DURATION, D	DURATION OF HOSPITALIZATION, D	CAUSES OF DEATH
1	2	F	NNRD at 4/10 Prematurity of 34 SA	Catheter	6	Ceftriaxone Gentamicin	5	-	-	5	Sepsis
2	1	M	NNRD 3/10	Blood culture	5	Ceftriaxone Gentamicin	5	Imipenem- Amikacin	3	8	Sepsis
3	1	F	DRNN at 3/10 Prematurity of 34 SA	Blood culture	7	Ceftriaxone Gentamicin	7	-	-	7	Prematurity sepsis
4	4	M	Neonatal jaundice incompatibility ABO	Blood culture	4	-	-	-	-	4	Sepsis
5	2	M	DRNN at 4/10 Prematurity of 29 SA	Blood culture	6	Ceftriaxone Gentamicin	7	Imipenem- Amikacin	1	8	Sepsis Prematurity
6	13	M	Neonatal jaundice	Urine	1	Ceftriaxone Gentamicin	10	-	-	7	Sepsis

Abbreviations: ATB: antibiotics; NNRD: neonatal respiratory distress.

The resistance to carbapenems was confirmed by the determination of the MICs on agar medium by E-test.

The antibiotics tested were ticarcillin, piperacillin, piperacillin-tazobactam, ceftriaxone, ceftazidim, cefotaxim, cefoxitin, aztreonam, cefepime, imipenem, meropenem, ertapenem, amikacin, tobramycin, gentamicin, colistin, ciprofloxacin, trimethoprim-sulfamethoxazole, fosfomycin, and tigecycline.

The identified strains showed the same pattern of antibiotic resistance: these isolates of *K pneumoniae* retained sensitivity to third-generation cephalosporins, aminoglycosides, and fluoroquinolones. Resistance to ertapenem was consistent with constant resistance to  $\beta$ -lactamase inhibitors (amoxicillin clavulanic acid, piperacillin tazobactam, and ticarcillin



**Figure 1.** Antibiogram of the strain of *Klebsiella pneumoniae*.

clavulanic acid). The strains remained susceptible to imipenem but exhibited an intermediate sensitivity to fourth-generation cephalosporins (Figure 1 and Table 2).

A modified Hodge test for detection of carbapenemase production and synergistic tests with and without inhibitors were performed for all isolated strains with resistance to ertapenem.

The Hodge test performed on all isolates was positive (Figure 2). Synergy tests with and without ethylenediaminetetraacetic acid (EDTA) were negative.

The genotyping by polymerase chain reaction (PCR) study was applied for detecting the carbapenemase *bla*<sub>OXA-48</sub> genes, *bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>NDM</sub> using special primers.

Thereby, this genotypic study of the epidemic strain allowed to demonstrate the presence of the *bla*<sub>OXA-48</sub> gene in *K pneumoniae* (Figure 3).

The alert was triggered from the first isolated case by the microbiology laboratory; the additional precautions were then set up by the service as well as the reinforcement of the bio-cleaning, in collaboration with the operational hygiene team (OHT) and the local committee against nosocomial infections.

#### Local management of the epidemic

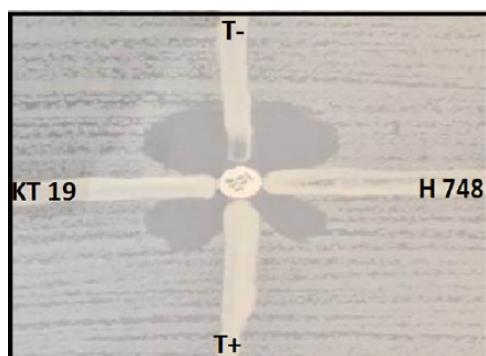
At the level of the neonatal resuscitation, various actions were carried out following this observation:

- Establishment of systematic geographical isolation of infected patients
- Surveillance of cases by OHT
- Audit of the complementary hygiene precautions of contact type set up by the OHT
- Increased awareness of the use of hydro-alcoholic solutions to improve the observance of hand hygiene

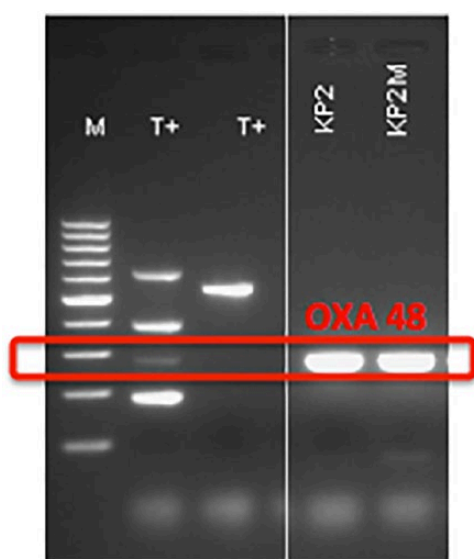
**Table 2.** Antibiogram with MIC of carbapenemase-producing *Klebsiella pneumoniae* isolates.

ANTIBIOTICS	MIC	PROFILE	ANTIBIOTICS	MIC	PROFILE
Ticarcillin	>64	R	Amikacin	≤2	S
Piperacillin	>64	R	Tobramycin	≤1	S
Ticarcillin-clavulanate	>64/2	R	Gentamicin	≤1	S
Piperacillin-tazobactam	>64/4	R	Colistin	≤0,5	S
Ceftriaxone	1	S	Tigecycline	1	S
Cefepime	≤1	S	Fosfomycin	≤16	S
Imipenem	2	S	Aztreonam	≤1	S
Meropenem	2	S	Trimethoprim-sulfamethoxazole	>4/76	R
Ciprofloxacin	≤0,13	S	Ceftazidime	1	S

Abbreviation: MIC, minimal inhibitory concentration.



**Figure 2.** Positive modified Hodge test for epidemic strains.



**Figure 3.** Result of the PCR with identification of the OXA-48 gene of the *Klebsiella pneumoniae* strain. PCR: polymerase chain reaction.

- Sensitization of the cleaning team to the reinforcement of bio-cleaning and to the mastery of the hygiene of the environment
- Screening for multiresistant bacteria carriage in all newborns admitted and transferred to the service
- Pediatric emergency department surface samples that were negative
- Compliance with the isolation measures of the infected patients, the reinforcement of the specific hygiene measures, and the sensitization of the personnel made it possible to put an end to the epidemic without closing the unit

## Discussion

Bacteremia due to carbapenemase-producing enterobacteriaceae is an emerging medical problem. The management of this entity is complicated by the difficulty of identifying resistance profiles and therapeutic options that remain limited.<sup>6</sup>

This study highlighted the impact of hand-carried transmission in the dissemination of strains and the occurrence of epidemics. Furthermore, it sheds light on the therapeutic

difficulties for the management of these newborns infected with a strain carbapenemase producing.

The particularity of the epidemic strain isolated in neonatology is that it presents an isolated resistance to ertapenem; the other carbapenems were susceptible on the antibiogram. Ertapenem appears to be a good marker for most carbapenemase-producing strains. Indeed, the MICs of ertapenem are generally higher than the MICs of other carbapenems.

The detection of strain-producing carbapenemase in clinical samples is essential which is based on a careful analysis of the results of sensitivity examinations that can be acquired with automated systems on liquid environments or agar diffusion tests of antibiotic disks. Automated systems may not detect all kinds of carbapenemase producers and contradictions might appear.<sup>7</sup> Detecting the carbapenemase producers was based only on MIC values of ertapenem and may also miss the specificity.<sup>8</sup>

OXA-48 carbapenemase producing strains have a very low activity with respect to the third and fourth generation of cephalosporins. However, these latter are rarely a therapeutic option because other  $\beta$ -lactamases such as ESBL are frequently associated and that is the case of this epidemic strain of *K pneumoniae* which preserved a constant sensitivity to cephalosporins of the third generation in vitro.

Molecular techniques remain the reference for recognizing and differentiating the carbapenemases. Most of the techniques are based on single or multiple PCR and can be followed by sequencing if necessary for the accurate identification of carbapenemase.<sup>9,10</sup>

The modified Hodge test combined with synergy tests with and without inhibitors may provide good phenotypic orientation and may be a good alternative to the unavailability of molecular confirmation.

The basic disadvantages of molecular technologies based on the detection of carbapenemase genes are their cost, the requirement of trained technicians, and the inability to detect new carbapenemase genes.<sup>8</sup>

Neonatal resuscitation is a unit at risk for the emergence and spread of multi-resistant antibiotic strains. Many contributing factors may be involved such as immaturity of the newly born's immune system and exposure to multiple invasive producers (urinary catheters, intubation, mechanical ventilation). The pressure of broad-spectrum antibiotic therapy, length of stay prolonged, immunosuppression, and hand-transmitted transmission of these germs caused this high potential for dissemination.

Bacteremias caused by the epidemic strains of *K pneumoniae* have been associated with a poor prognosis with a mortality of all infected newborns despite the in vitro susceptibility of these strains to third-generation cephalosporins and aminoglycosides, antibiotics that have been administered in combination as a probabilistic antibiotic therapy in these newborns.

The immune fragility of these children, the significance of the state of sepsis, and the importance of the bacterial

inoculum can demonstrate this deadly evolution and the non-response to the third-generation cephalosporins and antibiotics found sensitive *in vitro*.

The therapeutic options in case of infection proven by carbapenemase-producing bacteria are extremely limited, hence the importance of preventing and controlling infections with these bacteria.<sup>11</sup> In all cases and whenever possible, combined therapy is preferable to prevent the emergence of new resistances. Depending on the antibiogram of the bacterium, a combined treatment including an aminoglycoside (gentamicin, amikacin) may be considered.

Two old antibiotics have been revived with the appearance of carbapenemases: colistin (polymyxin E) and fosfomycin. The combination of carbapenems with colistin, tigecycline, and fosfomycin remains recommended for the treatment of infections due to carbapenemase-producing strains with MICs below 16 mg/L.<sup>12</sup>

The support of the patients at a high risk of carriage or infection with carbapenem-resistant strains should additionally include a strict respect to the standard precautions, the setting up of an accurate isolation, and hygiene measures as well as the detection of patients who are carriers of multi-resistant bacteria.<sup>5</sup> The healthy carriers can serve as a reservoir for the transmission of strains, hence the importance of screening. In the case of carriage's screening or infection with carbapenem-resistant enterobacteria in a hospitalized patient, screening must be carried out among all patients with an epidemiological link with the index patient (hospitalized patients in the same unit).<sup>13</sup>

The right use of antibiotics is also a part of the fight against the prevalence of carbapenemases. Decreasing the prescription of an antibiotic has shown a complete reduction to the prevalence of germs which are resistant to it.<sup>14</sup>

The oversight and the strict implementation of infection control measures is essential to prevent a quick spread of epidemics in health care facilities.<sup>15</sup>

The surveillance and strict implementation of infection control measures is essential to prevent the rapid spread of epidemics by these multi-resistant or pan-drug resistant isolates in health care facilities.<sup>15</sup>

## Conclusion

The production of carbapenemase in *K pneumoniae* is an emergent resistance mechanism that should be suspected and identified to provide targeted therapy and allow the implementation for the measures limiting its spread. Mastering the spread of an epidemic is based on the implementation of a local policy for the control of epidemics with multi-resistant organisms, insisting on

the good observance of the complementary and standard precautions, on the fact to be able to have a ratio carers/patients adapted, and on the collaboration between the unit and the OHT.

## Author Contributions

TL - Writing the article.

AAH - Realization of the Polymerase chain reaction bibliographic search.

BF - Synthesis of Clinical Records.

SN - Validation of clinical records.

MFMR - Validation of the article.

SN - Correction and validation of the article.

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