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Incidence of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* among Patients and in the Environment of Hassan II Hospital, Settat, Morocco

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Abstract

Aim: The aim of the current study is to determine: (1) the prevalence of extended-spectrum *β*-lactamase-producing *K. pneumoniae* (ESBL-Kp) isolated from clinical samples and a hospital environment in Hassan II Hospital (Settat, Morocco); (2) the associated risk factors of ESBL-Kp infections; (3) the link between clinical and environmental isolates. Methods: During the study period (April 2010 to March 2011), all patients infected and hospital environment sites contaminated by *K. pneumoniae* were considered as the potential study population and environmental site. The clinical data were collected to identify risk factors for ESBL carriage of *K. pneumoniae* infection. Screening of ESBL-and carbapenemase-producing isolates was performed by using a double-disk synergy test and the modified Hodge test, respectively. ESBL-Kp isolates were tested for the presence of genes encoding *β*-lactamases and were investigated by PCR. The clonal relationship between

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ESBL-producing isolates was analysed by ERIC- and REP-PCR method. Results: The overall prevalence of ESBL-Kp among clinical and environmental *K. pneumoniae* isolates was 35.13% (13/37) and 4.04% (4/99), respectively. The main risk factors for carrying ESBL-Kp were renal disease (46.15%), recent surgery (53.84%), previous hospitalisation (76.92%), and the presence of many invasive devices (53.84%). All ESBL isolates were multidrug resistant. The bla_{CTX-M} group1and bla_{SHV} (70.58% for each) were the most prevalent followed by bla_{TEM} (52.94%). Thirteen strains expressed at least two *bla* genes. One isolate was positive in the modified Hodge test and was a bla_{OXA-48} producer. ERIC and Rep-PCR methods revealed an epidemic clonal dissemination of these isolates. Conclusion: The emergence of OXA-48 carbapenemase, endemic clonal dissemination and multi-drug resistance of ESBL-Kp isolates in our institution is highly alarming.

1. Introduction

Klebsiella pneumoniae is an important pathogen known to cause nosocomial infections, such as urinary tract as well as respiratory tract infections and septicemia. Moreover, the infections caused by this isolate can be extended by the hospital environment. *K. pneumoniae* persists to be the major ESBL-producing organism isolated in the hospital settings all over the world. Infections due to ESBL-producing *K. pneumoniae* (ESBL-Kp) increased mortality in hospitalised patients [1]. Risk factors for the acquisition of ESBL-Kp involve mechanical ventilation, the length of hospital stay especially in the intensive care units ICU, and previous use of antibiotics, identified to be the most prevalent risk factors for ESBL-producing bacteria [2]. Various class of β-lactamases have been described in ESBL-Kp, within the class A ESBL types, including the TEM, SHV and CTX-M, is the most extensive and clinically relevant [3]. The ESBL are commonly plasmid encoded. The Plasmid usually carry genes encoding resistance to other antimicrobial agents, it is frequently responsible for ESBL production [4]-[6]. In Moroccan hospitals, the existence of ESBL-producing organisms has been reported and their prevalence varies from 8% to 51% [7] [8]. Nevertheless, various epidemiological factors combined with ESBL-producing strains acquire to be described. Nosocomial infections caused by *K. pneumoniae* may lead to a potentially life-threatening condition [2] [9]. Knowledge of local epidemiology is required to define the practical treatment for patients with a hospital infection.

The present study aims to determine the rate of ESBL-Kp isolated from clinical samples and hospital environment sites, and to find the relation between them. We have also determined the resistance of ESBL strains to other antimicrobial groups and analysed risk factors associated with the rate of infections by ESBL-producing strains.

2. Materials and Methods

2.1. Design, Setting and Recruitment

Patients' recruitment took place between April 2010 and March 2011 at Hassan II Hospital. This hospital is situated in Settat city (72 km on the north side of Atlantic Casablanca, Morocco). The hospital structure is composed of five major departments (emergency and intensive care, mother child, surgery, medicine and medico-technical) with a capacity of 302 beds. The Hassan II Hospital is a referral hospital for a population of approximately 1 million in the Settat area.

In this study, patients admitted to Hassan II Hospital were required to have nosocomial *K. pneumoniae* isolates coming from clinical specimens during their hospital stay. According to the Center for Disease Control (CDC) criteria, the diagnosis of nosocomial infection was established. Previous hospitalisation was defined as an admission at Hassan II Hospital or another hospital within 30 days prior to the current admission. The origin of the isolate was accepted as nosocomial if the strain was isolated more than 48 hours after hospitalisation.

Microbiological specimens were collected when the attending physician suspected infection based on systemic

signs (unexplained fever, chills and hypotension) and/or local signs (purulent tracheal aspirates in mechanically ventilated patients, purulent urinary drainage, or pus or pain at a vascular catheter insertion site). Microbiological specimens were collected as recommended by the CDC.

2.2. Data Collection

Data involve basic demographic characteristics (age, sex, pre-infection hospital stay, and nosocomial origin), in addition to the comorbid diseases (surgical intervention; renal, respiratory and central nervous diseases; along with many more). The data involve also intensive care unit admission, previous hospitalisation, recent surgery, and duration of hospital stay.

2.3. Environmental Samples

Environment samples were collected from the equipment and surfaces of the medical, intensive care unit (ICU), surgery, paediatric and maternity wards. Sampling of contaminated surface can be done with cotton-tipped swabs (Copan Diagnostics, California, USA), moistened with nutrient broth before use then placed in 1.5 mL of the same solution. Samples were then vortexed before being incubated overnight at 35˚C.

2.4. Culture Methods

After being incubated overnight, the nutrient broth samples were recorded to show either growth or no growth, based on the presence of a turbid solution. The samples showing growth were then plated onto Mac Conkey (MAC) agar and incubated overnight at 35˚C. Any colonies on MAC agar were identified using conventional methods, as well as the commercial identification kit API 20E (Biomerieux, Marcy l'Etoile, France).

Antimicrobial drug susceptibility testing for *K. pneumoniae* isolates was performed according to the disk diffusion method on Mueller-Hinton agar medium, and the results were interpreted according to the recommendations by the Clinical and Laboratory Standards Institute (CLSI) [10]. The following antimicrobial agents (Bio-Rad, Marnes-la-Coquette, France) were tested: amoxicillin/clavulanic acid (20/10 µg), cephalothin (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), imipenem (10 µg) and ertapenem (10 µg). ESBL production was screened using a double disk with a synergy test between third-generation cephalosporin (cefotaxime, ceftazidime) or a monobactam (aztreonam) disks, placed at a distance of 30 mm (centre to centre) from the amoxicillin/clavulanic disk as previously described [11]. This test is considered as positive if we noted a zone of inhibition between the cephalosporin antibiotics and amoxicillin/clavulanic disk, after 24-h incubation.

The detection of ESBL among AmpC hyper-producers was assessed by a double combination disk test on cloxacillin (250 µg/mL) agar [11]. The standard strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as a negative and a positive control of ESBL production. All the isolates with a reduced susceptibility to an imipenem disk (diameter of zones of inhibition, <22 mm) or an ertapenem disk (diameter of zones of inhibition, ≤21 mm) were screened for the production of carbapenemase by using the modified Hodge test according to the recommendations of the CLSI [10].

2.5. Molecular Studies

2.5.1. Preparation of DNA Template for PCR
Total DNA was extracted by the boiling method by suspending a few colonies of overnight culture of *K. pneumoniae* isolates growing on Luria Bertani agar (Bio-Rad) in 500 µL of DNase-and RNase-free water (Invitrogen, Paisley, UK). The suspension was boiled at 100˚C for 10 minutes in a thermal block (Polystat 5, Bioblock Scientific, France), then centrifuged at 19,000 x g for 5 minutes. An aliquot of 1 µL of the supernatant was used as the DNA template for PCR.

2.5.2. Detection of *β*-Lactamase-Encoding Genes
K. pneumoniae isolates were screened by PCR for the following *β*-lactamase-encoding genes: bla_{CTX-M} group 1, bla_{CTX-M} group 9, bla_{CTX-M} group 2, bla_{TEM}, bla_{SHV}, bla_{OXA-48}, bla_{NDM}, bla_{KPC} and bla_{VIM} as described previously [12]. The known *β*-lactamase–producing strains *E. coli* U2A1790 (CTX-M-1), *Salmonella* spp. U2A1446 (TEM-1

and SHV-12), *E. cloacae* U2A 2242 (KPC-3) and *K. pneumoniae* U2A 2016 (VIM-1) were used as positive controls. The *E. coli* $K_{12}J_5$ strain was used as a negative control. PCR products were detected by electrophoresis on a 1.5% agarose gel (FMC Bioproduct, Rockland, USA) containing 1 µg/mL of the ethidium bromide (BET) and detection by UV illumination. The gel picture was captured with an Olympus digital camera, and analysed using Digi-Doc-it software (UVP, Upland, USA).

2.5.3. Genotyping by ERIC-PCR and REP-PCR

The ERIC and REP (Enterobacterial Repetitive Intergenic Consensus and Repetitive Element Palindromic)-PCR, was performed using the primers ERIC-2 and Rep-2 [13]. The amplification reaction was carried out in a 25 µL volume containing 2.5 mM of $MgCl_2$, 50 µM of each deoxynucleoside triphosphate, 0.3 µM of the selected primers, 5 µL of the DNA template and 1.5 U of *Taq* DNA polymerase (Promega, Madison, Wisconsin, USA) Aliquots (10 µL) of each PCR product were subjected to electrophoresis on a 1.5% agarose gel.

2.6. Statistical Analysis

To identify variables associated with ESBL-Kp, a risk-factor analysis was performed using a case-control study format. Demographics and hospitalisation variables of ESBL-Kp case patients were compared with patients with non-ESBL-Kp isolates. Data were entered into a database using SPSS 20.0 for Windows (SPSS Inc, Chicago, USA). The χ^2 test and the independent samples *t* test were used for categorical and continuous variables, respectively. A stepwise multivariate logistic regression was lead to examine the association of risk factors controlling for potential confounders. The logistic model included all variables for which a *p*-value of < 0.1 was obtained in the multivariate analysis. A *p*-value of <0.05 was considered as significant.

3. Results

3.1. Patient Characteristics

Between April 2010 and March 2011, 37 of 192 (19.27%) infected patients were included in this study and had *K. pneumoniae* isolates. These patients were 62.16% female and 37.84% male (F: M ratio was 1.64).Thirteen patients presented ESBL-Kp infection (35.13%) and 24 patients had non-ESBL-Kp infections. The main epidemiological data of the infected patients by ESBL-Kp and non-ESBL-Kp isolates are shown in **Table 1**. The duration of hospitalisation for patients infected by ESBL-Kp isolates was 15.30 (±14.23) days, and 9.17 (±12.09) days in non-ESBL-Kp isolates (*p* > 0.05). A significant proportion of patients positive for the presence of ESBL-Kp isolates were males when compared to patients infected by non-ESBL-Kp isolates (*p* = 0.003), and the mean age of patients infected by ESBL-Kp isolates was higher than that of the patients infected by non-ESBL-Kp isolates (*p* = 0.02). The wards were sorted according to the number of reported cases in each ward: the surgical ward (53.84%), the intensive care unit (30.76%) and the medical ward (15.38%).

The statistical analysis showed that there was no significant difference between patients infected by ESBL-Kp and non-ESBL-Kp isolates in the infected regions. In both patients groups, the most common site of infections was the urinary tract, which yielded growth mostly in patients infected by ESBL-Kp isolates (**Table 1**).

Several factors, such as a previous hospitalisation, renal diseases, admission to surgical ward and urinary tract infection, were found to be significantly associated with an increased risk for acquisition of ESBL-Kp isolates (**Table 1**).

3.2. Rates and Distribution of Environmental Contamination

A total of 1000 samples were collected during the study period. Out of these cultured samples, the growth of at least one bacterial type was observed in 26.5% (n = 265), of which 115 were from equipment (monitor, aspirator, ventilator, laryngoscope, etc.) and 150 were from the care cart (n = 60), mattress cover (n = 40) and washbasin (n = 50).

K. pneumoniae strains were identified in 99 (37.35%) cases, and according to the contaminated ward, the medical ward (52%) was heavily contaminated followed by the ICU, paediatric, surgery and maternity wards (**Table 2**). Among these isolates, 4 (4.04%), ESBL-Kp isolates were identified from the surgery ward and the contaminated surfaces were as follow: washbasin (n = 1), mattress cover (n = 1) and care cart (n = 2).

Table 1. Clinical background characteristics of patients infected and not infected with ESBL-Kp.

Variable	All isolates n = 37(%)	ESBL-Kp isolates n = 13 (%)	Non-ESBL-Kpisolates n = 24 (%)	*p*-value
Demographics				
Age in years (mean ± SD)	43 ± 20	54.53 ± 21.36	37.6 ± 17.6	0.0265
Gender				
Female	23 (62.16)	4 (30.76)	19 (79.13)	0.005
Male	14 (37.83)	9 (69.24)	5 (20.87)	0.003
Sex-ratio	1.62	0.45	3.8	
Population				
Adult	19 (51.35)	4 (30.76)	15 (62.5)	NS
Elderly	17 (45.94)	8 (61.53)	9 (37.5)	NS
Child	1 (2.70)	1 (0.6)	0	NS
Previous hospitalisation	19 (51.35)	10 (76.92)	9 (37.5)	0.02
Duration of hospitalisation (mean ± SD)	11.32 ± 13.03	15.30 ± 14.23	9.17 ± 12.09	NS
Hospital admission ward				
Surgical	9 (24.32)	7 (53.84)	2 (8.33)	0.004
Maternity	14 (37.83)	0	14 (58.33)	0.0003
Medical	6 (16.21)	2 (15.38)	4 (16.66)	NS
ICU	8 (21.62)	4 (30.76)	4 (16.66)	NS
Infected regions				
Respiratory samples	4 (10.81)	2 (15.38)	2 (8.33)	NS
Urine samples	22 (59.45)	9 (69.23)	13 (54.16)	NS
Blood samples	2 (5.40)	1 (7.69)	1 (4.16)	NS
Vaginal samples	5 (13.51)	0	5 (20.83)	NS
Others	4 (10.81)	1 (0.6)	3 (12.5)	NS
Comorbidities	20 (54.05)	12/13 (92.30)	8/24 (33.33)	NS
Diabetes mellitus	5 (13.51)	0	5 (20.83)	NS
Renal disease	6 (16.21)	6 (46.15)	0	0.0007
Anaemia	1 (2.70)	1 (0.6)	0	NS
Cerebro-vascular accident	1 (2.70)	1 (0.6)	0	NS
Status epilepticus	3 (8.10)	3 (23.07)	0	NS
Others	5 (13.51)	2 (15.38)	3 (12.5)	NS
Reason for hospitalisation				
Childbirth	11 (29.72)	0	11 (45.83)	0.003
Urinary tract infection	7 (18.91)	3 (23.07)	4 (16.66)	NS
Surgery	11 (29.72)	7 (53.84)	4 (16.66)	0.027
Bronchopulmonary infections	3 (8.10)	2 (15.38)	1 (4.16)	NS
Polytrauma	3 (8.10)	1 (7.70)	2 (4.16)	NS
Toxaemia	2 (5.40)	0	2 (8.33)	NS
Type of invasive device				
Intubation + ventilation	5 (13.51)	1 (7.70)	4 (16.66)	NS
Central venous catheter	25 (67.56)	5 (38.46)	20 (83.34)	0.005
Intubation + urinary catheter+ ventilation	3 (8.10)	3 (23.07)	0	0.03
Urinary catheter+ central venous catheter	4 (10.81)	4 (30.76)	0	0.01

NS: Not significant.

Table 3. Antimicrobial susceptibilities of clinical and environmental ESBL-Kp strains isolated in Hassan II Hospital, Settat, Morocco.

Antimicrobial agents	Resistance ratios, % (no. resistant strains)							
	Clinical strains				Environmental strains			
	All	ESBL (n = 13)	Non-ESBL (n = 24)	*p*-value	All	ESBL (n = 4)	Non-ESBL (n = 95)	*p*-value
Amoxicillin/clavulanic acid	81.08 (30)	100 (13)	70.83 (17)	0.03	40.40 (40)	100 (4)	37.89 (36)	0.02
Ciprofloxacin	45.95 (17)	92.3 (12)	20.83 (5)	<0.0001	40.40 (40)	100 (4)	37.89 (36)	0.02
Ceftriaxon	35.14 (13)	100 (13)	0	<0.0001	4.04 (4)	100 (4)	0	<0.0001
Ceftazidime	35.14 (13)	100 (13)	0	<0.0001	4.04 (4)	100 (4)	0	<0.0001
Cefotaxime	35.14 (13)	100 (13)	0	<0.0001	4.04 (4)	100 (4)	0	<0.0001
Cephalothin	81.08 (30)	100 (13)	70.83 (17)	0.03	40.40 (40)	100 (4)	37.89 (36)	0.02
Cefoxitin	16.22 (6)	38.5 (5)	4.16 (1)	0.01	13.13 (13)	25 (1)	12.63 (12)	NS
Imipenem	0	0	0	-	0	0	0	-
Ertapenem	0	0	0	-	1.01 (1)	25 (1)	0	0.04
Aztreonam	35.14 (13)	100 (13)	0	<0.0001	0	0	0	-
Amikacin	0	0	0	-	0	0	0	-
Gentamicin	32.43 (12)	84.6 (11)	4.16 (1)	<0.0001	32.43 (28)	100 (4)	25.26 (24)	0.005
Cotrimoxazole	48.65 (18)	61.5 (8)	41.66 (10)	NS	48.65 (38)	50 (2)	37.89 (36)	NS

NS: Not significant.

Table 4. Characteristics of ESBL-producing *K. pneumoniae* detected among clinical and environmental isolates.

Code	Date of isolation	Sex/age	Source	Ward	*β*-lactamase type	Genotype of ESBL producer		
						ERIC-PCR[*] profile	REP-PCR[*] profile	Clone
Kp2	24/01/2011	M/70	Urine	Surgical	CTX-M, SHV, TEM	I	1	CK1
Kp4	01/02/2011	-	Mattress cover	Surgical	CTX-M, TEM			CK1
Kp6	18/01/2011	M/36	Urine	Surgical	CTX-M, SHV			CK2
Kp7	01/02/2011	-	Care cart	Surgical	CTX-M, SHV			CK2
Kp8	01/02/2011	M/70	Urine	Surgical	CTX-M	II	2	CK2
Kp10	01/02/2011	-	Care cart	Surgical	CTX-M, SHV			CK2
Kp12	05/01/2011	M/58	Urine	Surgical	CTX-M, SHV			CK2
Kp13	04/03/2011	M/64	Pus	Surgical	SHV			CK2
Kp14	03/03/2011	-	Washbasin	Surgical	CTX-M,SHV,TEM,OXA-48	III	3	CK3
Kp15	09/03/2011	F/21	Urine	ICU	CTX-M, SHV, TEM			CK3
Kp16	09/03/2011	F/21	Protected distal bronchial	ICU	SHV, TEM	VI	4	CK4
Kp17	09/03/2011	F/21	Protected distal bronchial	ICU	SHV, TEM			CK4
Kp1	28/09/2010	M/70	Urine	Surgical	CTX-M, TEM	ND		ND
Kp3	24/04/2010	M/71	Urine	Medical	CTX-M, TEM	ND		ND
Kp5	13/07/2010	M/65	Blood	ICU	CTX-M, TEM	ND		ND
Kp9	13/04/2010	F/74	Urine	Medical	SHV	ND		ND
Kp11	06/10/2010	M/68	Urine	Medical	SHV	ND		ND

ND: Not determined; * The ERIC-PCR and REP-PCR profiles for *K. pneumoniae* isolates are shown in **Figure 1**.
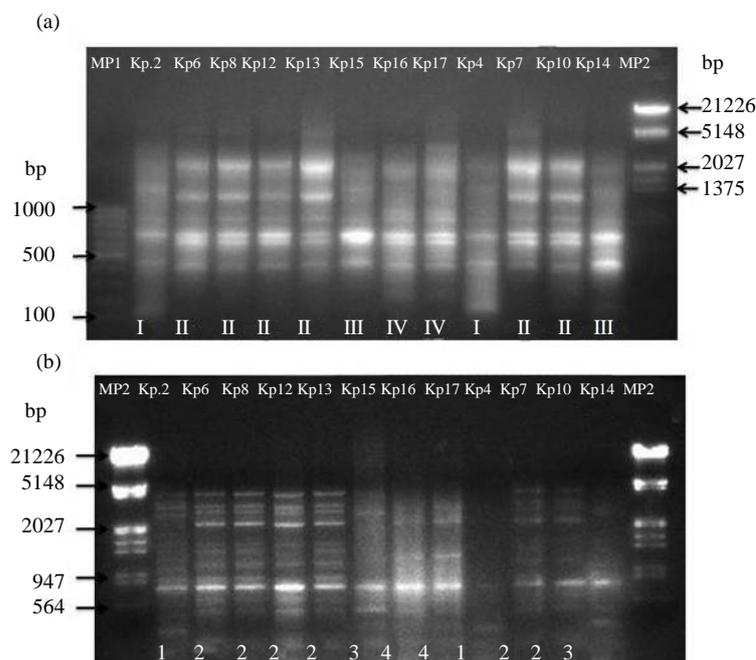
Figure 1. (a) Representative ERIC-PCR profiles for ESBL-Kpisolates. Lanes MP1: 100-bp DNA ladder, Lanes MP2: *Lambda* DNA was digested by *EcoRI/*Hind III, others lanes: isolates tested. (b) Representative REP-PCR profiles for ESBL-Kpisolates. Lanes MP2: Lambda DNA was digested by *EcoRI/*Hind III, others lanes: isolates tested.

represented by two strains, one isolated in the surgical ward from the environment (washbasin) and the other in ICU from a hospitalised patient. The clone CK4 presented the same band between two clinical strains isolated from the ICU.

4. Discussion

During the last few years, ESBL-producing *K. pneumoniae* represented the main cause of severe infections, not only in hospitals but also in community settings. The presence of ESBL restrains the activity of a wide number of antibiotics, leading to serious therapeutic difficulties with an important impact on the outcome of the patients [1]. The prevalence of ESBL-Kp has been increasing all over the world and has varied in different countries [1]. This study provides data from a Moroccan district hospital over a one-year period. The prevalence of ESBL-Kp was 35.13% for hospitalised patients. This rate was low compared to results reported in 2009 in Morocco, which showed a prevalence of 45% for ESBL-Kp [7]. However, this prevalence remains close to the that reported in some European and American hospitals (33% - 35%) [1].

The length of hospital stay mainly in ICU, mechanical ventilation, urinary catheterization, arterial catheterization, the use of a central venous catheter, and previous antibiotic treatment were found to be risk factors for the acquisition of ESBL-Kp infection along with many more [14] [15]. In this study, many risk factors for the acquisition of the ESBL-Kp infection were similar to those reported previously [14] [15]. The number of patients infected by ESBL producers receiving ventilator and catheter urinary, as well as intubation support, was higher than those infected by non-ESBL-Kp isolates. The presence of renal disease, recent surgery, male sex, and previous hospitalisation, were also found to be risk factors for the acquisition of ESBL-Kp infection.

The ERIC- and REP-PCR methods revealed four different profiles among the 12 ESBL-Kp isolates tested. The unit that was the most affected by the presence of ESBL-Kp isolates was surgery and its environmental sites (three clones). The spread of ESBL producers from one patient to another using equipment of the hospital environment has been reported many times [14]. This study confirms the serious role of the hospital environment in the transmission and spread of ESBL-producing infections in our hospital setting. Indeed, in many hospitals, the environmental contamination by ESBL-Kp is more frequent [14] [16] [17]. This could suggest that *K. pneumoniae*

has a higher capacity to persist in the environment than any other species, and it is able to form biofilms that may lead to its survival during long periods in the environment [18].

In general, the resistance patterns of ESBL-Kp studied here were alike to those commonly described in other studies, the ESBL producers were resistant to different antibiotic families, including fluoroquinolones, aminoglycosides and trimethoprim/sulfamethoxazole [1] [7] [8], which contributes to the variety and the persistence of multidrug-resistant-ESBL isolates and plasmids in hospital settings, resulting from the restraint of therapeutic options. In our finding, the rate of multidrug resistance was highly alarming (100%). Imipenem and amikacin were the most active among the antibiotics tested. It shows the real threat of this problem, particularly when we know that the increasing use of carbapenems, which have been widely used to treat serious infections with multidrug-resistant *K. pneumoniae*, has led to the appearance of carbapenem-resistant isolates via acquiring genes encoding carbapenem-hydrolysing enzymes.

In this study, one ESBL-Kp isolate was resistant to ertapenem, and was found to carry the bla_{OXA-48} gene; the latter was the most frequent gene reported in North African and Mediterranean countries [19] [20]. Recently, a nosocomial dissemination of OXA-48–producing *K. pneumoniae* isolates has been reported in Moroccan hospitals [21]-[23].

The screening of *bla* genes detected a diversity of *β*-lactamases, and the CTX-M type was the most common ESBL in our hospital. The predominance of CTX-M-15 is now common in Morocco, as in other countries, as a result of worldwide expansion [4] [5]. These high rates of CTX-M among total ESBL enzymes are most probably associated with the high mobilisation of the encoding genes, as it has been found that bla_{CTX-M} genes are mobilised to plasmids more frequently than other class A *β*-lactamases [24]. The genes encodedby the SHV family were detected in 70.58% of the isolates, and most enzymes of this group are ESBL, except for SHV-1 and SHV-11 [25]. However, the enzymes of this group cannot be confirmed to be ESBL-encoding genes, particularly in strains presenting more than one *bla* gene. On the other hand, the bla_{SHV-12} and bla_{SHV-27} genes were also detected in *K. pneumoniae* from Moroccan hospitals [21] [26]. The bla_{TEM} gene was identified in 47.05% of isolates, usually in association with SHV and/or CTX-M genes. Therefore, it was not possible to determine whether they were responsible for the ESBL phenotype; they could be non-ESBL-encoding genes associated with another gene expressing the extended-spectrum phenotype [25]. Conversely, the co-production of various ESBL by a unique strain has been a common result in many studies [5] [27] [28].

5. Conclusion

In conclusion, several clones of ESBL-Kp are widespread and are circulating both in the hospital environment and in patients. These strains harbour two or more *β*-lactamases genes along with the emergence of bla_{OXA-48} gene. A variety of risk factors associated with the occurrence of ESBL-Kp were detected. In summary, there is an urgent need to increase hygiene measures in order to limit the spread of these multidrug-resistant organisms in the hospital.

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