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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(3): 1921-1929. **OPEN ACCESS**

First Case Report of Isolation and Identification of *Escherichia coli* and *Klebsiella pneumoniae* Resistant to Beta-Lactamines from Clinical Sampling in Tunisia

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This study had for objective to identify nosocomial Enterobacteriaceae resistant to β-lactamines, which were isolated from different samples: blood culture, urine culture and Levy Tracheal Protected from the consultants or patients hospitalized Habib Thameur Tunis-Tunisia Hospital for four years from 2010 to 2013. A retrospective survey was made over 4 years (2010-2013). It involved 93 patients of pediatric surgery services and and the anesthesia resuscitation presenting an urinary and blood infection confirmed by the laboratory of microbiology of the hospital Habib Thameur of Tunis. The year 2012 was characterized by a significant increase of the patients affected by Escherichia coli β-lactamases with extended spectrum (E. coli BLSE) and *Klebsiella pneumoniae* β-lactamases with extended spectrum (K. pn. BLSE). We were a able identified the bacteria *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pn*.), of the readings of galleries and antibiogrammes. And further to test Hodge and E. test, we classified the strain *K. pn.* Carbapenemases (*K. pn. carba*), *K. pn.* metallo β-lactamase (*K. pn.* metallo), *K. pn.* β-lactamases and *E. coli* β-lactamases. The resistance of *E. coli* and *K. pn* in β-lactamines became worrisome so in a hospital environment as in community. Four multiresistant strains may be carriers of plasmids coding for resistance to β-lactam antibiotics have been detected. The succession of this mechanism of resistance involves a more rational use of β-lactamines, especially as a first-line treatment of urinary tract infections.

Keywords: Beta-lactam-resistant bacteria, Urinary tract infections, blood poisoning, respiratory infection

INTRODUCTION

Escherichia coli and *klebsiella pneumoniae* constitute constitute the major bacterial species involved in the urinary and respiratory related infections. The β-lactams are the drugs of choice for treating urinary tract infection owing to their low toxicity, bactericidal power, efficiency and low cost properties (Livermore, 1995). Their introduction in the therapy was rapidly followed by a description of resistance mechanisms (Tagajdid et al. 2010).

Opportunistic nosocomial bacteria that escape the effect of β-lactams are threaten public health, causing serious and untreatable infections, some deadly, especially in hospital settings. These

resistances are connected to an accumulation defect in contact with the target (or the PLP penicillin-binding proteins); this occurs in case of antibiotic impermeability (or efflux), modification of PLP or production of inactivating enzymes called β-lactamases (Galleni et al. 1995, Walsh, 2008). These resistance mechanisms must be detectable by antibiotic susceptibility testing to allow the use of the most effective molecule (Robin et al. 2012) to help the antibiotic prescription and optionally monitoring the evolution of their resistance.

Human transmission is the main vector for passage of resistant bacteria from one individual to another. The environment plays only a marginal role. The transmission of resistance from animal to man certainly plays an increasing role through food. The history of antibiotic therapy or hospitalization are a major factor in antibiotic resistance for urinary tract infections (De Moüy et al. 2001).

The abuse of antibiotics is not without consequences, several studies have reported increased resistance on the African continent that are the subject of a national or international monitoring because of their high potential for dissemination and spectrum activity. Some rates are similar to those reported in some European and American countries.

In 2000 , five africians country namely: Tunisia , Morocco , Algeria, Senegal and Ivory Coast , were part of the initiative “Inspear” (International Network for the Study and Prevention of Emerging Antimicrobial Resistance) , initiated by the Center for Disease Control in Atlanta , United States , and concerns 39 countries from all continents (Dosso et al. 2000).

The City biologist can be integrated into a networks for monitoring resistance to antibiotics through its role in the diagnosis and treatment of community infections. This monitoring should take into account historical and medical information of patients.

In this work we proposed to identify certain pathogenic enterobacteria, multi-resistant to β-lactam antibiotics isolated from different cyto-bacteriological samples from hospitalized patients in the hospital Habib Thameur in Tunisia.

MATERIALS AND METHODS

Patients and methods

Place and period of the study

The present work is a retrospective study that was conducted over four year period ranging from 2010 to 2013. It involved 93 patients with urinary or respiratory infection or sepsis confirmed by the microbiology laboratory of the Habib Thameur hospital.

Inclusion Criteria

An Cyto-Bacteriological Urine Exam (CBUE) (urine culture), a Endotracheal Aspirate Cultures (EAC) and a blood culture were performed during 4 years (2010-2013) to the 93 patients examined or hospitalized at the Habib Thameur hospital, clinically suspected suffering from urinary or respiratory infections or septicemia. An appropriate culture environments were used with respect to each sample collected to identify specific pathogens.

Methodology

Urine, blood or bronchial secretion samples were immediately transferred to the microbiology laboratory. The samples were examined under a microscope by means of gram staining method. To ensure that the microorganism in question is of a Gram-negative bacillus, there was used a staining kit consisting of four reagents: Gentian Violet (violet color), Lugol's solution (a solution of elemental iodine and potassium iodide in water), a 90% Alcohol and Fuchsine (pink color).

The samples gave results Gram-negative and were then inoculated on different agar CLED agar, MacConkey agar and blood agar and incubated at 37 °C for 24 hours (Betty et al. 1998). Colonies that showed a lactose fermentation on the MacConkey agar and CLED agar were purified and identified according to their circular morphology: pink colonies, pink to red on the MacConkey agar and yellow colonies on the CLED agar.

The isolates were identified by biochemical reactions for example indole, urease and citrate test, catalase enzyme, Voges-proskaur reaction, H2S and oxidase test (Holt et al. 1994). Afterwards, additional tests for bacterial identification were established according to the method in use in the laboratory i.e. the API20E gallery (BioMérieux). The Antibiograms of the gram-negative bacilli were made by the diffusion method on an agar plates (Mueller Hinton agar) in accordance with the recommendations of the Committee on Antimicrobial Susceptibility Testing of the French Society for Microbiology (CA-SFM) (Tagajdid et al. 2010). The Antibiotics tested were: amoxicillin, amoxicillin-clavulanic acid, ticarcillin, piperacillin, piperacillin-tazobactam, cefalotin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, ertapenem, aztreonam, amikacin,

gentamicin, nalidixic acid, norfloxacin, ciprofloxacin, colistin, tetracycline, cotrimoxazole, fosfomycin and nitrofuran. The diameter of the inhibition zone was measured and the isolates were classified as "resistant", "Intermediate" and "sensitive" on the standard basic graphic.

Detection of the producers of β-lactamases was performed by the disc diffusion method based on synergy detection between amoxicillin disk and six other disks: ceftazidime, ertapenem, aztreonam, imipenem, cefepime and cefotaxime.

The study of sensitivity to gentamicin brings one more element reflecting the multi-drug resistance. Indeed, the association between ESBL production and resistance to gentamicin seems natural.

The "Hodge Test"

A disk of ertapenem (ETP) is applied at the center of a Mueller-Hinton (MH) box previously inoculated with a wild *E.coli* strain (sensitive to carbapenems) in order to obtain a culture confluent and a diameter in the area sensitivity around the ETP. The strains to be tested are applied to the agar in the form of streaks deposited from the ETP disk to the edge of the box. After one night at 37 ° C, the deformation of diameter at the intersection between a ridge and the *E. coli* culture indicate the presence of a hydrolysis carbapenems by the strain tested (Lee et al. 2001). This method, athough easy to perform, in no way prejudges the identification of the β-lactamase involved. In addition, the test is sometimes difficult to interpret.

The "Epsilometer" Or E-Test

It is a complementary test to the Hodge's test; a strain suspension spread testing k. pn. was made on Mueller-Hinton agar (Bio-Mérieux). It is based on the use of a graduated band divided into two: one is soaked in a solution of a given imipenem concentration and the other in a solution of a given (imipenem + ethylene diamine tetra-acetic) concentration deposited on the surface of the agar. The incubation is achieved for 24 h at 37 °C under aerobic conditions. By simply reading CMI and the diameters of the inhibition halos, it is possible to identify the strain. If the ratio: CMI imipenem (mg/l) / CMI "imipenem + ethylene diamine tetra-acetic acid" (mg/l) is ≥ 8 it can be concluded that the tested germ is a *k. pn.* metallo..

RESULTS

Evolution of nosocomial infections caused by β-lactamine multi-resistant Enterobacteriaceae in Habib Thameur Hospital:

In the late 90s, the world of bacteria, especially the Enterobacteriaceae family, testified the emergence of new microorganisms that have a great resistance power to the family of the most used antibiotics: the family of β–lactam antibiotics. These multi-resistant Enterobacteriaceae (*K. pn.* Metallo, *K. pn.* Carba, *K. pn.* ESBL and *E. coli* ESBL) are present in the various hospital services, but they are even more common in ICUs where cases are more critical (RAISIN 2006).

In this preliminary work, and based on statistics stored in the hospital Habib Thameur, we built the histogram below (Figure 1), where we have shown trends in the numbers of patients with nosocomial infections during the last three years. Thus, the strain "K. pn. metallo" was born in 2013. The strain *E. coli* ESBL and *K. pn.* ESBL, exist since 2010. The year 2012 was characterized by a significant increase in patients with *E. coli* ESBL and *K. pn.* ESBL. During the same year, the stain *K. pn.* carba appeared.Fig 1

Identification of nosocomial Enterobacteriaceae

The following table summarize the results of identification tests performed:

In this study, we reported some urinary infections with *E. coli* or *k. pn.* and respiratory infections and sepsis by *k. pn.* This result agrees with that of Iroha et al. (2009) who indicated that *E. coli* is one of the most common bacteria that can cause infection in humans and especially an urinary tract infection.

These *E. coli* and *K. pn.* were identified by biochemical reactions (Table 1 and Table 2). In case of treatment failure in special clinical situations mentioned above, antibiotic sensitivity should be known. We can detect exceptional resistance to β-lactam in *E. coli* and *K. pn.* by agar diffusion method or appreciate antibiotic susceptibility using systems such as Hodge test, the E-test or ATI20E gallery and the box of synergy. For this, we performed the susceptibility of each strain (figure 2) and finish the identification. Antibiograms of these strains are presented in Table 3. The study of the antibiotic susceptibility of bacteria difficult to culture poses technical problems due to their slow growth and the need to use enriched media. Their profile of usual sensitivity to the various antibiotics families are known, but it was recommended to report the sensitivity profile of strains unusually resistant, that have been isolated by random not standardized diffusion in which the use of automates is not possible.

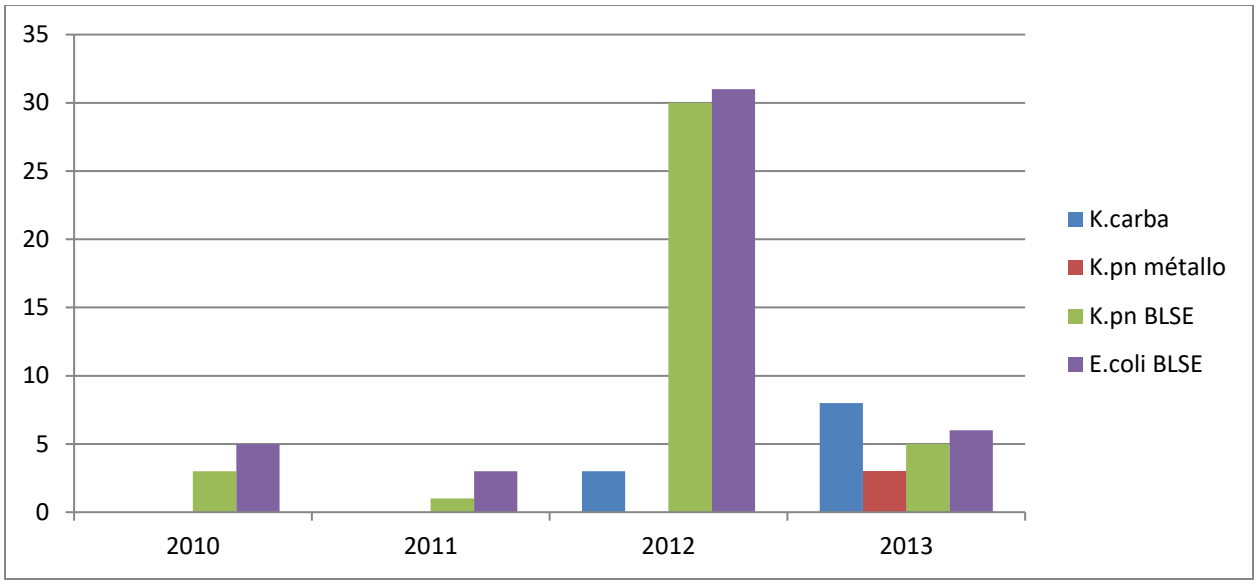

Figure 1. The results of a statistical nosocomial infections caused by multidrug-resistant Enterobacteriaceae from 2010 until 2013 at the Habib Thameur Hospital.

Table 1. Reading the Various Tests Mentioned

Middle of the gallery		**Urea**		**Hajna**			**Citrate**	**Mannitol**	
The physico-chemical criteria		Urease	Indole	clearance of the gas and degradation of glucose	Lactose	H_2S	clearance of the CO2	Degradation of mannitol (reduction of nitrate it nitrite)	mobility
coloring	Test +	The coloring turns pink	Presence of a brown ring	Presence of air bubbles in the agar and the pellet turns yellow	Slope Remains red	Appearance of black precipitate at the base	The color of the medium turns blue	The coloration of the medium remains red	Mobile bacterium: there is distribution of colonies in the middle
	Test –	The coloringre mains yellow	Absence of brown ring	Absence of air bubbles and the nerve remains red	The slope turns yellow	No black precipitate	The coloring of middle remains green	The coloring turns yellow	Motionless bacterium: colonies remain located just instead of the sowing

Table 2. Comparison of Physico-Chemical Parameters of Different Germs Brought into Play.

Middle of the gallery	Urea		Hajna			Citrate	Mannitol	
The physico-chemical criteria	Urease	indole	glucose	Lactose	H_2S	Clearance of CO2	Degradation de mannitol	Mobility
K.pn	+	-	+	+	-	+/-	+	-
K.pn.carba	+	-	+	+	-	+/-	+	-
E.coli	-	+	+	+	-	-	+	+/-
E.coli.BLSE	-	+	+	+	-	-	+	+/-
K.pn.BLSE	+	-	+	+	-	+/-	+	-
K.pn. metallo	+	-	+	+	-	+/-	+	-

+ : Result ; – : No Result

Table 3. Sensitivity and inhibition zone (Mm) of different antibiotic drugs used against clinical isolates of *Escherichia Coli* and *Klebsiella Pneumoniae*

Germ		*Escherichia coli* BLSE		*Klebsiella pneumoniae* BLSE			
Sample		Urine		Urine		Blood	
Antibiotic (4 µg)	Threshold	Resultat	Diameters	Resultat	Diameter	Resultat	Diameter
Amoxicilline	16-21	Resistant	8.00±2.00	Resistant	6.33±0.57	Resistant	7.00±1.00
Amoxicilline+Acide Clavulanique	16-21	intermediate	20.33±0.57	intermediate	16.66±0.57	Resistant	8.66±2.51
Ticarcilline	22-24	Resistant	10.33±3.78	Resistant	6.66±0.57	Resistant	7.66±1.52
Piperacilline	16-20	Resistant	8.00±1.73	Resistant	9.66±4.72	Resistant	6.00±0.00
Piperacilline+ Tazobactam	17-21	Sensitive	25.00±1.00	intermediate	18.66±1.52	Resistant	12.33±5.50
Cefalotine	12-18	Resistant	6.00±0.00	Resistant	7.66±1.52	Resistant	9.00±2.64
Cefoxitine	15-22	Sensitive	27.00±0.57	Sensitive	28.66±1.52	Resistant	11.00±1.00
Cefotaxime	23-26	Resistant	14.00±3.00	Resistant	15.66±5.85	Resistant	9.33±3.05
Ceftazidime	21-26	Resistant	19.00±1.00	Resistant	17.66±1.52	Sensitive	28±1.00
Cefepime	21-24	Resistant	17.66±2.08	Resistant	17.66±1.15	Resistant	8.33±2.08
Imipeneme	17-24	Sensitive	30.33±1.52	Sensitive	29.00±1.00	Resistant	14.00±1.00
Ertapeneme	26-28	Sensitive	30±1.00	Resistant	9.33±2.08	Resistant	8.00±2.00
Aztreonam	21-27	Resistant	19.00±1.00	Resistant	16.00±1.00	Sensitive	6.00±0.00
Amikacine	15-17	Sensitive	23.00±1.00	Sensitive	23.00±1.00	Sensitive	19.33±1.52
Gentamicine	16-18	Resistant	13.33±1.52	Resistant	8.66±2.51	Resistant	7.33±1.52
Acide Nalidixique	15-20	Resistant	7.33±1.15	Sensitive	22.33±1.52	Resistant	6.66±0.57
Norfloxacine	22-25	Resistant	7.00±1.73	Sensitive	29.33±0.577	Resistant	7.337±1.15
Ciprofloxacine	22-25	Resistant	7.00±1.00	Sensitive	32.00±1.00	Resistant	7.00±1.73
Colistine	15-15	Sensitive	18.66±0.57	Sensitive	18.33±1.52	Sensitive	23.00±6.00
Tetracycline	17-19	Sensitive	24.33±1.52	Resistant	8.00±1.73	Sensitive	22.33±1.52
Cotrimoxazole	13-16	Resistant	6.00±0.00	Resistant	8.33±2.08	Resistant	8.33±3.21
Fosfomycine	14-14	Sensitive	28.66±2.08	Sensitive	19.00±1.73	Resistant	12.66±0.57
Nitrofurane	15-15	Sensitive	25.00±1.73	Sensitive	20.33±2,51	Resistant	6.33±0.57

Antimicrobial susceptibility by the disc method is however usable with discs containing 4 mcg. An

inhibition diameter greater than 20 mm is generally obtained with the sensitive strains; there is no inhibition when the strains are resistant. This study cannot be properly made only by the methods of determining the MIC of a strain with a diameter of inhibition less than 20 mm by dilution techniques and perhaps by E test that has proven its validity in our case.

In conclusion, following the combination of readings galleries and antibiograms, the germs are found to be: Escherichia coli and *Klebsiella pneumoniae*.

After tests of identification mentioned above, additional tests were performed. These tests concerned the enterobacteria showing a multi-resistance to almost all antibiotics involved.

The ringed antibiotic discs are the β-lactams antibiotic. According to the antibiograms of the strains involved, we took bacteria with alarming resistance to β-lactams to perform the following tests:table 3

Urinary tract infection is a frequent pathology. E. coli is the major bacterial species involved in this type of infection. In this study, susceptibility testing to antibiotics that have been made to *E.coli* isolates associated with urinary tract infections, showed have shown that fosfomycin, nitrofuran, “piperacillin + tazobactam”, cefoxitin, imipenem, ertapenem, amikacin, colistin and tetracycline were the most effective antibiotics against E. coli ESBL. Imipenem and ertapenem have shown a maximum efficiency with respective inhibition diameters of 32 and 31 mm. Amoxicillin + clavulanic acid had an intermediate effect against these same E. coli ESBL isolated.

On the other hand, these isolates of E. coli ESBL have showed a strong resistance to norfloxacin and ciprofloxacin by having inhibition diameters of 6 mm relative to the thresholds between 22 and 25 mm. Thus, amoxicillin, ticarcillin, piperacillin, cefalotin, cefotaxime, ceftazidime, cefepime, aztreonam, gentamicin, nalidixic acid and cotrimoxazole have not shown any effect against E. coli ESBL isolated.

These results agree with those reported by Kader et al. which found that the E. coli showed high levels of resistance to amoxicillin (Kader et al. 2000), but does not agree with the results found in 2011 by Al-Jiffri et al. (2011), the antibiotic ciprofloxacin was the most effective against E. coli isolates. Similarly, our results agree with those reported by Rosa et al. who stated that the antimicrobial agents with the highest levels of activity against Gram-negative bacilli was amikacin, what was reserved for hospital use, while the ciprofloxacin and nitrofurantoin showed acceptable levels of activity (Rosa et al. 2001)..

With tests using different antibiotics from those used in our antibiogram, Larabi et al. identified from urine of consultants and patients hospitalized in the University Hospital “La Rabta -Tunis” from January 1996 to April 1998, strains of phenotypes *E.coli E*SBL and *K. pn.* ESBL resistant to gentamicin, tobramycin and amikacin, respectively 0.9% and 4.7%. In addition those K. pn. Have shown resistance to pefloxacin (6.1%) and ciprofloxacin (2.1%), (Larabi et al. 2003). In another study in Egypt, multi-resistance of E. coli strains to antibiotics in food was higher compared to those of clinical origin (Aly Marwa et al. 2012).

Klebsiella pneumoniae is a saprophytic and commensal Enterobacteriaceae of the human digestive tract which is responsible for mainly respiratory and urinary, community and nosocomial, infections. The antibiotic susceptibility testing that have been made to the *K. pn.* isolates associated with urinary tract infections have shown that cefoxitin, imipenem, amikacin, nalidixic acid, norfloxacin, ciprofloxacin, colistin, fosfomycin and nitrofuran were the most effective antibiotics against *K. pn.* ESBL. Unlike, the isolates *E. coli* ESBL, the most effective antibiotics were norfloxacin and ciprofloxacin with respective inhibition diameters of 29 and 33 mm against *K. pn.* ESBL. “Amoxicillin + clavulanic acid” and “piperacillin + tazobactam” had an intermediate effect against these same *K. pn.* ESBL.

Furthermore, amoxicillin, ticarcillin, piperacillin, cephalothin, cefotaxime, ceftazidime, cefepime, ertapenem aztreonam, gentamicin, tetracycline and cotrimoxazole showed no effect against K. pn. ESBL isolated. Unlike the *E. coli* ESBL isolates, the *K. pn.* ESBL were resistant to both antibiotics tetracycline and cotrimoxazole with an inhibition’s zone diameter of 6 mm as compared to the respective thresholds of 17-19 and 13-16.

The antibiograms made *with K. pn.* isolated form blood showed that these were sensitive to the antibiotics: ceftazidime, aztreonam, amikacin, colistin, tetracycline and fosfomycin. By against these bacteria isolated were resistant to amoxicillin, “amoxicillin + clavulanic acid”, ticarcillin, piperacillin, “piperacillin + tazobactam”, cefalotin, cefoxitin, cefotaxime, cefepime, imipenem, ertapenem, aztreonam, gentamicin, nalidixic acid, norfloxacin, ciprofloxacin, cotrimoxazole, and fosfomycin and nitrofuran.

The increase in the number of extended-spectrum β-lactamase producing Enterobacteriaceae (ESBLs) resulted in misuse of

carbapenems in many countries, leading to the emergence of resistance to these antibiotics, especially in K. pn. The resistance to carbapenems in Enterobacteriaceae is mainly due to the production of metallo-beta-lactamase.

In our study, the specificity of the Hodge-test with the ETP disk (inhibition diameter = 21 mm) and Muller-Hinton agar for the blood culture from the ICU, gave a positive test with the streak of strain K. pn. tested that touched the ETP disk, that is why it was ranked *K. pn.* Carba.

The Committee on Antimicrobial Susceptibility Testing of the French Society for Microbiology gave no specific recommendations about the choice of media or critical diameters; common interpretation criteria for all other germs are therefore also applied to these bacteria. The antibiogram does not seem to be the best method to assess the antibiotic susceptibility of difficult-culture bacteria. The methods of determining the MIC by dilution in solid medium seems best suited Although the conditions of their realization are not yet standardized (Sanson-Le-Pors and Raskine, 1995).

Via the E-test, the metallo-β-lactamase, resulted in a positive synergy test was revealed in the strain K. pn. tested; that is why she was ranked *K. pn.* metallo β-lactamases.

Through the effects of synergies between the antibiotics used in the synergy box, this test made it possible to classify the strains *K. pn.* ESBL and E*. coli* ESBL.

DISCUSSION

In 1998, L. Martinez-Martinez discovered the first quinolone plasmid resistance determinant in K. pn. which was a protein encoded by the resistance gene that was named Qnr A1. Since then, new variants resulting from amino-acid substitutions have been identified (Qnr A2, Qnr B1, Qnr S1 ...), (Nordmann and Mammeri, 2007). In E. coli, we find Qnr A1, Qnr B3, Qnr B4 and Qnr S1. Moreover, in 2007, Guessennd et al. found that the prevalence of the association "Qnr-ESBL" was 27% on average for all enterobacteria, which underlines the possibility of a co -selection of these two mechanisms of plasmid resistance due to Qnr-type gene determinants and those of ESBL. The same study reported that for E. coli, the percentage of ESBL strains with Qnr genes was 31% (Guessennd et al. 2008). Among the 168 strains of Klebsiella pneumoniae producing β-lactamase isolated in 1998 by Ben Rejeb et al. (1998), 29 (17.26%) have expanded spectrum.

In 2005, a second mechanism of plasmid resistance to ciprofloxacin was identified. It is a variant of the acetylase on 6-isoform Ib which confers low-level resistance to ciprofloxacin and norfloxacin by enzymatic acetylation (Robicsek et al. 2005). In addition, during the period 2005-2010, Mezghani et al. (2012) were able to isolate from 93 patients, 121 colistin-resistant Enterobacteriaceae strains, of which *K. pneumoniae* constituted the most frequent species (60.2 %), followed by E. cloacae (26.9 %) and E. coli (12.9 %). Most of strains were isolated from urine (80.6%), 11.8% in the blood, 6.4% from the tracheo-esophageal tract and 0.9% from catheters (Mezghani et al. 2012).

The progression of acquired resistance of enterobacteriaceae to β-lactams is the consequence of the selection pressure due to the wide use of β-lactams. Moreover, these resistances acquired because of their plasmid determinism, have a great power of dissemination (CCASFM 1996). In 1991, in the Tunisian neonatal service, Hammami et al. (1991) found 21 cases of gastroenteritis due to infections even with other bacteria Salmonella wien resistant to β-lactams.

In 2013, Barguigua et al. Have demonstrated a higher prevalence of ESBL-producing urinary E. coli in a Moroccan community, which increased in triplicate over a two-year period by 1.3% to 4.1% in 2011. This proves that ESBL-producing strains continue to spread in this community.

The excessive use of large spectrum antibiotics in human and veterinary medicine with a lack of attention to laboratory screening for ESBLs produced by isolates can lead to the emergence and spread of these ESBL-producing strains. In addition, person-to-person transmission within family members via fecal products could contribute to the spread of ESBL as reported by Rodriguez-Bano et al. (2008). In addition, the presence of ESBL in cattle, poultry, dogs and cats stresses that food-producing animals and domestic animals may act as a reservoir for the transmission or acquisition of ESBL genes or ESBL production for the Enterobacteriaceae in the community (Brinas et al. 2005).

CONCLUSION

E. coli and K. pn. Can cause urinary, blood and pulmonary infections because of their specific pathogenicity factors. These germs have natural resistances and acquired resistances due to the selection pressure of certain widely used antibiotics. For all these reasons, currently the treatment of these infections, even in the community, must be prescribed on the basis of an antibiogram. Thus, regular monitoring of antibiotic resistance and knowledge of hospital ecology is

essential to support the choice of effective empirical treatments adapted to local epidemiology, limiting the emergence and spread of multidrug resistant strains, and Preserve the most active molecules..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

We wish to thank most sincerely Pr. Ridha Khalifa her assistance during our studies

AUTHOR CONTRIBUTIONS

AB designed, supervised the project and reviewed the manuscript. ED, MD and MAB collected the samples and performed experiments and data analysis. All authors read and approved the final version.

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