DOI:10.21276/sjpm.2017.2.1.1

Saudi Journal of Pathology and Microbiology
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2518-3362 (Print)
ISSN 2518-3370 (Online)

Original Research Article

Prevalence of resistant *Escherichia coli* strain isolated from community acquired urinary infection in university hospital HASSAN II of Fez, Morocco

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Abstract: The urinary tract infection is one of the most common infections acquired in community and hospital. The emergence of the acquired resistance of bacteria to antibiotics remains an important issue relevant to antimicrobial strategies. The Purpose of this paper is to review the frequency of isolation and the evolution of the resistance to antibiotics of the tree strains of Escherichia coli causing community urinary tract infections. It is a retrospective study made in the laboratory of microbiology of the university hospital center HASSAN II of Fez, concerning germs isolated from 5287 positive Bacteriological examination of urine (BEU) collected between 2010 and 2016 and diagnosed in consulting externs. Enterobacteriaceae were responsible for 84 % of the community urinary tract infections. The women were more touched (sex ratio F/H = 1, 66). *Escherichia coli* dominated the epidemiological profile (62 %). The antibiotic resistance of the tree strains of *E. coli* isolated highlighted rates of resistance to amoxicillin (64 %), to ciprofloxacine (59 %), to association amoxicillin-acid clavulonic (46 %), to sulfamethoxazole-triméthropime (42 %), to gentamicine (25 %), and to amikacine (14 %). The number of tree strains of *E.coli* resistant to third generation cephalosporins by production of B-lactamases with widened spectre"ESBL -producing" was 258, that is an average frequency of 5.8 % of all the enterobacteriaceae isolated. No resistance to imipeneme was registered for the tree strains of *E. coli* isolated whitch means that the sensibility to imipeneme is of 100 %. All these results showed an increase of the multiresistance of *Escherichia coli* in community environment which could be due to the excessive use of antibiotics in the medical domain. A regular surveillance of the resistance to antibiotics is essential to define effective and appropriate therapeutic strategies, limiting the emergence and the scattering of the multiresistant tree strains**.**
Keywords: urinary tract infection - Escherichia coli - Antibiotics – surveillance.

INTRODUCTION

In Morocco and worldwide, the rational use of antibiotics is a major concern. This increase concerned particularly the consumption of broad spectrum penicillin and quinolones. This high consumption is probably leading to high prevalence of the resistances to antibiotics of numerous pathogenic bacteria [3-5], although we cannot assert it with certainty for every sort (species) and every antibiotic. Today, the emergence of resistant multi-bacteria (BMR) is very worrisome not only in hospital but also in community environment where *E.coli* is the most frequent (40 in 70 %) of germs isolated [6, 7]. In this paper, the objective is to estimate the rate of antimicrobial resistance of the tree strains of *E.coli* isolated of community urinary tract externs patients and to change the antimicrobial strategies depending to the local epidemiology.

MATERIAL AND METHODS

This retrospective study was conducted in the laboratory of microbiology in the university hospital HASSAN II from Fes (Morocco) during seven years from January 1st, 2010 to October 30th, 2016. This hospital has capacity of more than 900 beds and contains all the specialities.

Was included in this study all BEU coming from patients not hospitalized and sent by examination center.

For each patient was considered only one positif BEU.

The diagnosis of urine infection UI was based on the Kass criteria of [2]: white blood cells count (WBC) superior or equal to 10^4 elements / mL and bacteriuria superior or equal to 10^5 colony-forming units/mL. The thresholds of bacteriuria of 10^3 UFC / mL for the cystites with *Escherichia coli* were held.

METHODOLOGY

Urines was cultured and incubated in 37°C during 24-48 hours on a non-selective middle CLED ™ (Biomerieux). The identification of bacteria was

established according to the morphological, cultural, biochemical characters to know the gallery API20E (Biomerieux) or by method automated on Phoenix. The antibiogram was realized by the method of distribution of disks in non-selective middle Müller Hinton and interpreted after measure of the diameters of inhibition according to the committee of antibiogram of the French Society of microbiology (CA-SFM) and to CLSI guidelines [8].

The production of Extended Spectrum Beta-lactamas producing (ESBL- producing) was detected by double disc diffusion test. An amoxyclav (amoxicillin/ clavulonic acid) disc was placed in the middle of petriplate and third- generation cephalosporin discs were placed 20-30mm away from the central disc. An extension in the zone of inhibition, so-called keyhole effect, was considered positive for ESBL production (Figure1).

RESULTS

Over a period of seven years, 37235 samples of urines were collected from external patients with a regular progress of the number of demands of analysis going of 4120 in 2010 to 6934 in 2016. The number of BEU isolating a bacterium in a significant threshold was 5287 having a global rate of 14.2 % positivity (Table I).

The median age of the patients with positive culture was of 44 years with important variations according to the age bracket (Table II). The majority of the subjects were female with a sex ratio F/M of 1, 66.

The *Escherichia coli* dominated the etiology of urinary tract infections with a rate of 62 % of all the isolated bacteria and 75 % of grouped enterobacteriaceae (Table III). The enterobacteriaceae represente 84 % (n = 4441) isolates of positive urines.

Other species were isolated less frequently. They were essentially represented by *Klebsiella sp* (n=809; 15,3 %), *Enterobacter sp* (n = 365; 6.2 %), *Pseudomonas aeruginosa* (n=227; 4.3 %), *Staphylococcus saprohyticus* (n=195; 3,7 %), *Proteus sp* (n=132; 2,5 %), *Enterococcus faecalis* (n=100; 1.9 %), and *Citrobacter sp* (n=74; 1,4 %). The evolution (from 2010 till 2016) of the rates of resistance of *E coli* to all the tested antibiotics is presented in (Figure 2). The amoxicillin and the fluoroquinolones were the least active antibiotics with rates of sensibility not exceeding the 40 %. A loss of sensibility of the sulfamethoxazole trimethoprim was also noted going from 79 % in 2010 to 58 % in 2016. The resistance towards the C3G increased slowly, it remained lower than 10 %. Aminoglycosides preserved an excellent activity with a superior efficacy in 80 % and 90 % respectively for the gentamycine and the amikacine. The resistance to the association amoxicillin + clavulonic acid (AMC) has so labeled an increase from 32 % in 2010 to 46 % in 2016. Comparing 7 molecules most frequently tested during the period of study, the C3G and the aminoglycosides were the most active against of *Escherichia coli* (Figure 2). The frequency of isolation of the tree strains of *E coli* ESBL producing knew an increase, passing from 4.6 % in 2010 to 7.3 % in 2016 compared with all the tree strains of enterobacteriaceae isolated in urine tract infections (Table IV).

Resistances to antibiotics usual in the case of *E. coli* producers of ESBL producing were 85 % for the ciprofloxacine, 76 % for the SXT, 66 % for the gentamicine, 37 % for the amikacine and 0 % for the imipeneme.

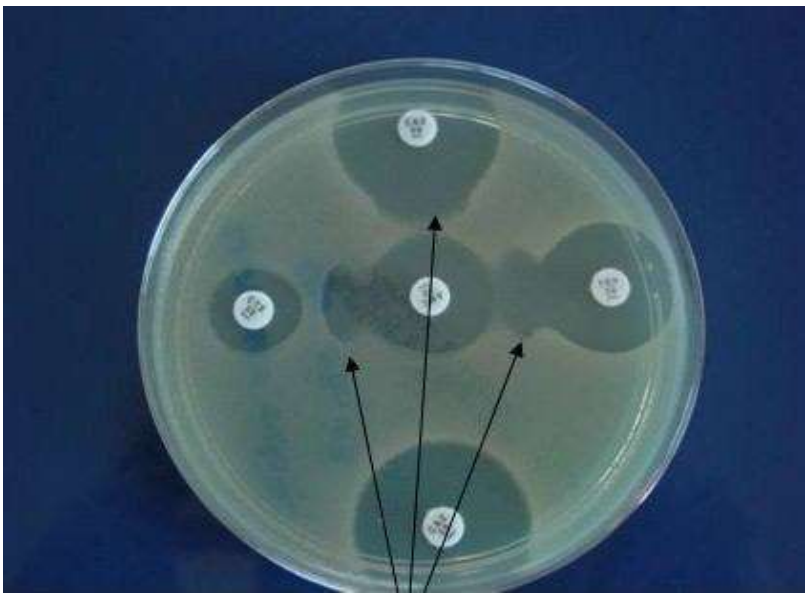

Fig-1: Synergy test

Table-I: Evolution of the frequency of isolation of E. coli in community urinary infections

	2010 n=636	2011 n=679	2012 n=727	2013 n=743	2014 n=806	2015 n=807	2016 n=849
E.coli	65.8%	63.3%	63.1%	60.7%	63.1%	62%	61%

Table-II: Frequency of E. coli urinary tract infections. Relationship between age and sex.

Age	Masculin	Féminin	Total
0-15ans	137 11.1%	178 8.7%	315 9.6%
15-30ans	273 22%	543 26.5%	816 24.9%
30-45ans	513 41.6%	497 24.3%	1010 30.8%
45-60ans	174 14.1%	458 22.4%	632 19.3%
>60ans	135 11.2%	370 18.1%	505 15.4%
Total	1232 37.6%	2046 62.4%	3278

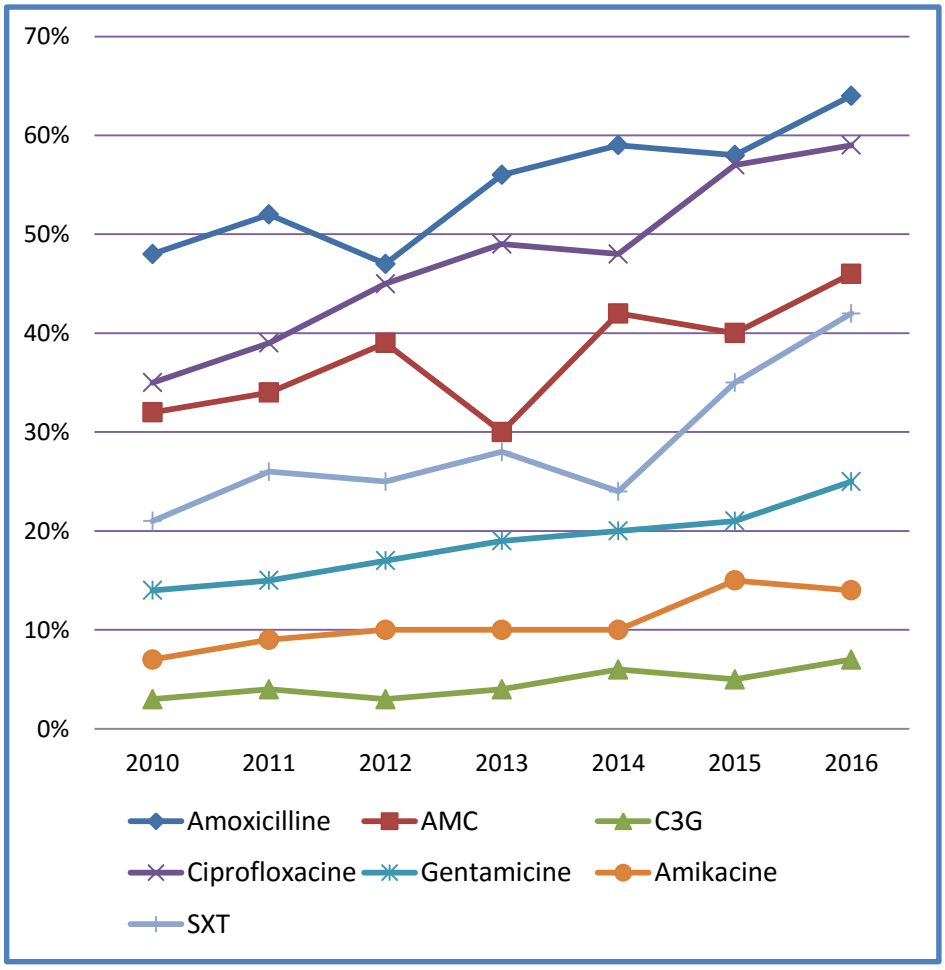

Fig-2: Evolution of resistances from 2010 to 2016 for E. coli

Table-III: Distribution of major bacterial species responsible for urinary tract infections from 2010 to 2016

Bactéries isolées	n (%)	2010	2011	2012	2013	2014	2015	2016
Escherichia coli	3278(62%)	419	430	459	451	509	500	510
Enterobacter sp	365(6.2%)	47	45	49	44	52	61	67
Proteus sp	132(2.5%)	14	17	19	16	21	20	25
Citrobacter sp	74(1.4%)	11	13	10	12	9	7	12
Klebsiella sp	809(15.3%)	95	98	105	127	130	126	128
Morganella. morganii	31(0.6%)	4	5	4	2	5	4	7
Pseudmonas aeruginosa	227(4.3%)	16	29	34	41	30	35	42
Providentia sp	5(0.1%)	0	0	1	2	1	0	1
Enterococcus faecalis	100(1.9%)	8	15	10	14	15	17	21
Enterococcus faecium	10(0.2%)	0	1	0	2	2	3	2
Staphylococcus saprophyticus	195(3.7%)	20	26	31	29	28	32	29
Staphylococcus aureus	21(0.4%)	2	0	5	3	4	2	5
Total	5247	636	679	727	743	806	807	849

Table-IV: Evolution of ESBL E. coli by Year

Années	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
Entérobactéries	534	565	605	619	685	697	736
***E coli* BLSE**	24	27	31	36	41	45	54
***E coli* BLSE en %**	4.6%	4.8%	5.1%	5.8%	5.9%	6.4%	7.3%

DISCUSSION

The urinary tract infection constitutes one of the most frequent community bacterial infections [10]. It imposes a considerable economic burden on the society. The profile of uropathogenes bacteria is dominated by enterobacteriaceae all around the world [9]. At the head of thread, we find *E coli* with a 65 % frequency. This can be explained by the ascending physiopathology of the UI as well as the strong colonization of the perineum by the enterobacteriaceae of digestive origin, and in particular *E.coli*, associated with the specific factors of pathogenicity of urine tract such as the bacterial adhesins capable of being bound in the urinary epithelium [11].

Our study confirms that women are more suseptible to develop a urinary tract infection because of specific contributing factors roams pregnancy and the most affected age between 15 and 65 years. Our resultsare in agreement with those published recently by Bourjilat and *et al*. [12]. These authors reported that, 75 % of the women and 25 % of the people presented a urinary tract infection due to E.coli, this infection presented 61 % at the people whose age is between 15 and 65 years, 27 % for the age bracket 15 years and 12 % for the people whose age is upper to 65 years.

This study confirms the alarming nature of the resistance evolution in *E coli*. Indeed, it shows an evolution of the clinical strains towards the resistance with respect to almost all the antibiotics. The main risk factor for consensual resistance is prior exposure to antibiotics. It is currently recognized that the use of an antibiotic or a class of antibiotics is the cause of the progression of bacterial resistance to this antibiotic. Thus the resistance rates observed are closely related to the quantity of antibiotic used [13, 5, 15].

In our study, a high aminopenicillin resistance level of 64% was recorded. Even higher levels of resistance to amoxicillin, up to 75%, have been reported in other studies [16, 17]. This increase in resistance to amoxicillin has been noted globally leading to the elimination of this molecule from the list of probabilistic treatments recom- mended in the UI [18].

The acquisition of resistance to AMC, a very prescription antibiotic in Morocco, is a global phenomenon reported at very variable rates [19]. In our study, resistance to AMC was 46% versus a resistance rate of 13.7% in the city of Eljadida. At the level of the city of Rabat, resistance to amoxicillin-clavulonic acid combination was 50% in consultant patients.

Third generation cephalosporins, on the other hand, are very active on this bacterium, with very low resistance rates (7%) which are consistent with those of many countries in the Mediterranean [20].

The main mechanism of resistance is the acquisition of a wide-spectrum beta-lactamase (ESBL) of plasmid origin degrading all beta-lactams except carbapenems [21]. This phenomenon affected 16% of the whole of the isolated strains. At the level of the Marrakech region, the production of ESBL by *E. Coli* uropathogens increased from 2% in 2008 to 6% in 2012 [22]. In a case-control study, the risk of ESU to E coli ESBL was shown to be related to the notion of a urinary abnormality, hospitalization during the last 12 months or previous antibiotic therapy [23].

Fluoroquinolones occupy a privileged place among the molecules prescribed in the treatment of urinary infections and in particular in the probabilistic

treatment of uncomplicated acute cystitis of the woman. Like any other antibiotic, the relation between increase of the consumption of fluoroquinolones and the increase of the bacterial resistance in these molecules is not to be any more demonstrated. The world epidemiological situation of the resistance of *E. Coli* with fluoroquinolones remains variable with resistance rates of 10% in the United States [24] and 50% in China [25].

The SMX-TMP, is a major antibiotic in the treatment of urinary tract infections, in particular the low cystites of the woman. In our study, the prevalence of the resistance slightly increased keeping an average rate of 40 % resistance. Certain older studies place it between 20 and 40 % [26-29], pleading in favour of a bigger precaution in the prescription not documented of the cotrimoxazole.

No resistance to imipeneme was highlighted for the tree strains of E. coli identified, thatmeans a sensibility to imipeneme is of 100 %. However, the rational use of this molecule is compulsory to avoid the emergence of tree strains of coli E. producing of carbapenemases.

CONCLUSION

The increasing of the resistance of the community tree strains of E coli in antibiotics became a phenomenon alarming in Morocco. So, the distribution of the producing tree strains of ESBL is a major problem of the next decades. This incites to a reflection concerning the management of patients consulting for a urinary tract infection. The fight against this phenomenon crosses first of all by the control of the prescription of antibiotics and may be the questioning of certain probability treatments which are not any more adapted. On the other hand, these data must be completed by an active surveillance of the resistance to antibiotics on the whole of the territory so as to adapt better these recommandations to the local epidemiology of tree strains.

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