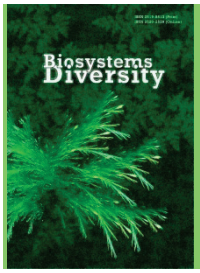

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Detection and prevalence of extended spectrum β-lactamases production among Enterobacteriaceae isolated from urinary tract infections

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Urinary tract infections (UTIs) by extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL-E) have become an important public health problem with a substantial economic burden, as they lead to more complicated infections, longer duration of treatment, and increases in patient mortality. A total of 1267 urine samples were collected from patients during the period of 2018 to 2020. The isolates were identified based on colony morphology and biochemical tests. Antimicrobial susceptibility testing was done by modified Kirby Bauer disc diffusion method. Resistant isolates to third generation cephalosporins were tested for ESBL production by double-disk synergy test (DDST), DDST methods on MH agar plates supplemented with cloxacillin and by confirmatory double disk test (DDT). 400 strains causing UTIs were identified and classified as members of Enterobacteriaceae. The results of antimicrobial susceptibility tests showed that 68.5% (274/400) of the Enterobacteriaceae isolates were multi-drug resistant (MDR) strains. The overall prevalence of ESBL-E was 59.5% (238/400). The highest frequencies of ESBL-E were observed in internal medicine devices (68/400) and the highest ESBL production was observed in *E. coli* (85/238). The ESBL producing isolates were significantly resistant to penicillins and cephalosporins compared to non-ESBL producers. However, those ESBL-E strains were sensitive to imipenem (80.2%) and nitrofurantoin (87.8%). High levels of MDR strains and ESBL-E in our study highlights the need for applying specific infection control measures, and accordingly we urge physicians to opt for specific strategies for regular surveillance of uropathogenic bacteria.

Keywords: antimicrobial susceptibility; urinary tract infections (UTIs); Enterobacteriaceae; extended-spectrum β-lactamases (ESBL) – producing; multi-drug resistance.

Introduction

Urinary tract infections (UTIs) caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae represents everywhere in the world, one of the main reasons for consultation, microbiological examinations and the overuse of antibiotics with, for the latter, the consequences on the cost of care and multi-resistant strains selection both in hospitals and in community settings (Dash et al., 2018).

Urinary tract infections (UTIs) are one of the commonly encountered infectious pathologies worldwide, with prevalence estimated to be around 150 million persons per year. A recent report reflects that more than 90% of UTIs are due to enteric Gram negative bacteria, mainly *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* (Hantalo et al., 2020). UTIs are treated with many classes of antibiotics, among them, the β-lactam antibiotics are the most commonly used because of their broad-spectrum activities and better safety profiles (Tekele et al., 2020). Many reports have suggested that the emergence of bacterial resistance is increasing due to the indiscriminate use of antibiotic prophylaxis and self-medication and these have resulted in selective pressure on the bacterial population with the emergence of resistant mutants (Benyagoub et al., 2021). Extended spectrum beta-lactamase (ESBL), metallo beta-lactamase (MBL) and Amp-C mediated beta-lactamases are some of the enzymes produced by Enterobacteriaceae and other non-lactose fermenters causing UTIs (Mechai et al., 2020). Over the last decade, various mechanisms of resistance of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) to antibiotics have been described, including target modification (expression of alternative penicillin-binding proteins), reduction in cell permeability through porin modification and efflux pump expression (Tooke et al., 2019). The extended spectrum β-lactamases (ESBL) belong to the group of enzymes produced by bacteria which have become resistant to extended-spectrum penicillin (or third-generation) cephalosporins and monobactams (aztreonam) but not to cephamycins (cefoxitin and cefotetan) and carbapenems (Kunishima et al., 2019). The recent functional classification schemes suggest the subdivision of this array of enzymes to: group 1 (class C) cephalosporinases; group 2 (classes A and D) broad-spectrum, inhibitor-resistant, and extended-spectrum β-lactamases and serine carbapenemases; and group 3 (class B) which comprises a heterogeneous group of zinc metalloenzymes (metallo-β-lactamases, or MBLs) (De Angelis et al., 2020).

The great capacity of Enterobacteriaceae to resist the main antibiotics used in antibiotic therapy, has led to a pessimistic conclusion about the effectiveness of our therapeutic arsenal in the treatment of BMR infections, in particular ESBL-producing Enterobacteriaceae (Tooke et al., 2019). However, epidemiological surveillance, as well as controlling the spread of these uropathogenic organism is a priority. This study was designed to investigate the prevalence, antibiotic resistance, and current distribution of uropathogenic ESBL-producing Enterobacteriaceae.

Material and methods

Samples collection, handling and processing. A total of 1267 urine samples were collected from patients during the period of 2018 to 2020, at referral hospitals in Tebessa province located in Northeast Algeria, namely: Tebessa Central Hospital (208 beds and 9 wards), Bekkaria Hospital (252 beds and 8 wards), Bir El Ater Hospital (170 beds and 7 wards), Cheria Hospital (150 beds and 5 wards), El Aouinat Hospital (132 beds and 5 wards) and Morsott Hospital (9 beds and 5 wards). The collected samples were labeled with clinical characteristics of the patients, including including age, gender, underlying diseases and results of physical examination, then all urine specimens were sent to the microbiological laboratory and were processed in 15–20 min for isolation and identification. The enrichment of urine samples is done by inoculating 1 mL of urine in 5 mL of nutrient broth, and then isolation, as for the other samples, was carried out on Mac Conkey agar according to the streak method. All isola-

tes were analyzed both by conventional bacteriological methods including colony morphology, pigmentation, isolation on cetrimide agar, oxidase test, and by Mini-API, a semi-automatized assay (bioMérieux, Marcy l'Etoile, France).

The study was carried out with ethical clearance and performed in accordance with relevant guidelines and regulations in Algeria. The patients' demographic data were collected using structured questionnaire.

Antibiotic susceptibility testing was performed on Mueller-Hinton agar (MH; BioMérieux, Marcy- l'Étoile, France) by standard disk diffusion method, using disk antibiotics (Liofilmchem) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST. European Committee on Antimicrobial Susceptibility Testing Recommandations. V. 2.0 September, 2019) guidelines against 22 antibiotics: amoxicillin (30 µg), ticarcillin (75 µg), ticarcillin-clavulanic acid (75/10 µg), piperacillin (100 µg), cephalexin (30 µg), cefoxitin (30 µg), amoxicillin-clavulanic acid (20/10 µg), ceftazidime (30 µg), cefexime (5 µg), cefepime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), fosfomycin (50 µg), nitrofurantoin (300 µg), tobramycin (10 µg), gentamicin (10 µg), amikacin (30 µg), erthapenem (10 µg), imipenem (10 µg). *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 strains were used as controls. The isolates were considered to be resistant if the diameter of the inhibition zone was inferior or equal to 22 mm for ceftazidime, 25 mm for ceftriaxone, and 27 mm for cefotaxime and aztreonam. The strains that showed resistance to at least one of the three cephalosporins were submitted to the ESBL detection tests.

Screening of multi-drug resistant (MDR) strains. MDR strains were screened according to the definition of Magiorakos et al. (2012): if the isolates were resistant to representative antibiotics of at least three different classes of antimicrobial agents, they were regarded as multi-drug resistant strains.

The double-disk synergy test (DDST) was carried out according to Jarlier et al. (1988). Third-generation cephalosporin disks, cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), ceftriaxone (CRO 30 µg), or aztreonam disk (ATM 30 µg) were placed 30 mm (center to center) from a central disk containing amoxicillin/clavulanic acid (AMC 20/10 µg). Strains that showed typical increase of the inhibition zone (>5 mm) between cefotaxime, ceftazidime, ceftriaxone or aztreonam and amoxicillin/clavulanic acid were identified as ESBL producers.

Double-disk test (DDT) was performed as described by Rahal, (2005). Disks of amoxicillin/clavulanic acid (AMC 20/10 µg) and third-generation cephalosporins (CTX 30 µg) were placed at 25 mm (center to center) on Mueller Hinton agar inoculated with the tested strain. After 1 h of incubation, the AMC disk was replaced by a CTX disk. The test was considered positive for ESBL production if the inhibition diameter of CTX disk applied after pre-diffusion of the AMC disk was ≥ 5 mm with respect to the diameter of CTX disk.

Confirmation of ESBL Producers by DDST methods on MH agar plates supplemented with cloxacillin. High-level expression of AmpC cephalosporinase may mask the presence of ESBLs, hence the double-disk synergy test on MH agar supplemented with cloxacillin (250 µg/mL) was performed. After overnight culture, test isolates were suspended to 0.5 turbidity using McFarland standards as a reference, and used to inoculate a Muellere Hinton agar plate containing 250 mg/mL cloxacillin. Discs containing ceftazidime, ceftazidime/clavulanic acid, aztreonam and cefepime were placed 2 cm from a disc containing amoxicillin/clavulanic acid. Cephalosporinase inhibition was considered positive when the ceftazidime zone diameter increased by 10 mm (Lin et al., 2012).

Statistical analysis. To determine if there was a statistically significant difference between the various parameters, t-test was performed using Minitab software (ver. 19.1.1). P-values were calculated using the Chi-square test. The differences were considered significant at $P < 0.05$.

Results

In the present study, a total of 970 Gram negative bacilli representing different colony morphologies were recovered from 1267 urine samples. Among these, 400 isolates were identified as members of the Enterobacteriaceae family. In relation to gender of patients with UTI, 228 (57%) were females and 172 (43%) were males (Table 1). The infection among the patients' age groups is ranged between 10 days and 90 years, the highest (50%, 200/400) occurrence was in young age adults (20–39 years), followed by middle aged adults (40–59 years) (26.5%, 106/400) and the lowest incidence was among old age adults (> 60 years). As shown in Table 1, internal medicine, internal surgery, gynecology, dialysis and maternity grouped the majority of identified cases with 25.0%, 16.2%, 15.7%, 15.2%, 10.5% respectively. Only a reduced number from the intensive care unit 7 (1.7%) and orthopedics device 1 (0.2%) were recorded. In addition, public hospitals establishment of Tebessa and Bekkaria shared frequencies ranged from 25.2% to 30.5% of total confirmed cases.

According to the distribution of strains in terms of species, *E. coli* with 66.3% (155/400) was the most frequent isolate followed by *K. pneumoniae* and *Enterobacter cloacae* with 12.1% (39/400).

The antibiotics sensitivity pattern amongst Enterobacteriaceae isolates (Fig. 1) revealed a high frequency of resistance to β-lactams ($P < 0.01$): amoxicillin (AMX) 89.2%, ticarcillin (TIC) 77.3%, ticarcillin + clavulanic acid (TTC) 72.5%, cephalexin (CL) 60.5% and cefixim (CFM) 45%. Regarding non-β-lactams antibiotics, resistance rates were high for ofloxacine (OF) 43.7% and cotrimoxazole (COT) 53.2%; significant for Fosfomycin (FOS) 41.5%, and low for gentamicin (GM) 21.0%, amikacin (AK) 11.0%, nitrofurantoine (NIT) 8.0%. Only imipenem (IPM) and nitrofurantoin (NIT) were effective against more than 87.8% and 92.0% of the isolates respectively.

Using the commonly used definition of multidrug resistance (MDR) as an organism being resistant to three or more classes of antibiotics, 68.5% (271/400) of the 400 isolates were identified as MDR (Table 2), among which *E. coli*, *Enterobacter cloacae* and *K. pneumoniae* contributed to 29.2% (117/400), 7.5% (30/400) and 7.0% (28/400) of the observed MRD respectively. The most effective antibiotics for MDR Gram negative bacilli were amikacin (97.1%), ertapenem (96.3%), and imipenem (95.5%). Only 9.75% (39/400) of the Enterobacteriaceae were susceptible for all antibiotics tested in this study.

From the total 400 Enterobacteriaceae isolated, 311 (77.7%) were found to be suspicious for ESBLs production based on the result of the DDST method. However, the DDST methods on MH agar plates supplemented with cloxacillin showed that 93 of Enterobacteriaceae were ESBL producers. Using the double-disk test (DDT), we confirmed that 238 (59.5%) of the suspected isolates were able to produce ESBL (Fig. 2). The distribution of uropathogenic ESBL-producers according to the species is shown in Table 2. Amongst the 238 ESBL-E positive strains we found that 45 (11.2%) were represented by *E. coli*, 20 (5.0%) by *K. pneumoniae*, then 13 (3.2%) by *E. cloacae*, and 7 (1.7%) by *Raoultella ornithinolytica*.

The 238 ESBL producers were commonly recovered from the internal medicine device 68 (17.0%), internal surgery 36 (9.0%), dialysis 37 (9.2%), gynecology 28 (7.0%) and infectious disease 25 (6.2%, Table 1). The distribution of ESBL producers based on gender indicates that women had a higher prevalence rate of 57% than men, 43% ($P = 0.006$). There was no significant difference between the gender distributions and the source patients. In terms of age, our results showed that it was found that the most frequently ESBL-producing Enterobacteriaceae were related to the patients aged 20–39 years (124/400, 31%) compared with other age groups. The count of ESBL-producing Enterobacteriaceae was different in the referral hospitals in Tebessa province. The count was highest in PHE Tebessa and PHE Bekkaria (15.7%, 63/238) followed by PHE Cheria (10.2%; 41/238) and EPH Ouinat (3.7%; 15/238), and lowest in PHE Morsott (0.7%; 3/238).

The pattern of antimicrobial susceptibility among 238 ESBL-E showed high prevalence of resistance to various antimicrobial agents (Fig. 1) revealed high frequency of resistance to β-lactams ($P < 0.01$): amoxicillin (AMX) 94.5%, ticarcillin (TIC) 91.6%, ticarcillin + clavulanic acid (TTC) 89.5%, cephalexin (CL) 86.9%, ceftazidime (CAZ) 89.1%, cefepim (FEP) 84.4%, aztreonam (ATM) 77.7%, cefotaxime (CTX) 73.5% and Cefexime (CFM) 85.7% and amoxicillin/clavulanic (AMC) 65.2%. Regarding non-β-lactams antibiotics, resistance rates were high for ofloxacin (OFX) 65.9% and nalidixic acid (NA) 55.6%; significant for cotrimoxazole (COT) 69.7% and ciprofloxacin (CIP) 56.3%; moderate for tobramycin (TOB) 55.2%, fosfomycin (FOS) 47.9%, and low for gentamicin

(GN) 37.3%, amikacin (AK) 18.5%, imipenem (IPM) 15.1% and nitrofurantoin (NIT) 12.2%.

Discussion

Urinary tract infection (UTI) is the second most common infectious disease throughout the world caused by a wide range of microbial pathogens. In the present study, a total of 970 Gram negative bacilli representing different colony morphologies were recovered from 1267 urine samples. Among these, 400 isolates were identified as members of the Enterobacteriaceae family. In relation to gender of patients, 228 (57%) were females and 172 (43%) were males (Table 1). This finding is supported by other studies reporting a higher rate of UTI prevalence in female patients compared to males (Zenati et al., 2018; Benyagoub et al., 2021). Furthermore, this result was expected, as women are more prone to developing UTI than males because females are more at risk of developing infection by uropathogens, which is due to their anatomical structure. About the distribution of infection among patients' age groups, our data showed that the most frequent uropathogens were related to the age groups 20–39 (50.0%) and 40–59 (26.5%) years old. These outcomes agree with many recent reports demonstrating that the incidence of UTIs was higher in adults than any other age category (Tekele et al., 2020; Gharavi et al., 2021).

Table 1
Distribution of ESBL-producing uropathogens from patients with UTIs according to gender, age, sex and type of hospital admission from UTIs

Characteristics		Total Enterobacteriaceae		Total of ESBL	
		number of isolates, n = 400 (100%)	P	number of isolates n = 238 (59.5%)	P
Gender n (%)	Female	228 (57.0)	0.006*	122 (30.5)	0.646
	Male	172 (43.0)		116 (29.0)	
	Sex-ratio	0.75	–	–	–
Age-years n (%)	1–19	71 (17.7)	0.002*	41 (10.2)	0.003*
	20–39	200 (50.0)		124 (31.0)	
	40–59	106 (26.5)		58 (14.5)	
	≥ 60	23 (5.7)		15 (3.7)	
Hospital admission ward n (%)	Internal medicine	100 (25.0)	0.000*	68 (17.0)	0.000*
	Internal surgery	65 (16.2)		36 (9.0)	
	Gynecology	63 (15.7)		28 (7.0)	
	Dialysis	61 (15.2)		37 (9.2)	
	Maternity	42 (10.5)		20 (5.0)	
	Infectious Disease	31 (7.7)		25 (6.2)	
	Pediatrics	19 (4.7)		19 (4.7)	
	Pneumo-phthisiology	11 (2.7)		1 (0.2)	
	Intensive care unit	7 (1.7)		4 (1.0)	
	Orthopedics	1 (0.2)		0 (0.0)	
Public hospitals n (%)	PHE Tebessa	122 (30.5)	0.000*	63 (15.7)	0.001*
	PHE Bekkaria	101 (25.2)		63 (15.7)	
	PHE Bir El Ater	87 (21.7)		53 (13.2)	
	PHE Cheria	49 (12.2)		41 (10.2)	
	PHE Ouinat	32 (8.0)		15 (3.7)	
	PHE Morsott	9 (2.2)		3 (0.7)	
Type of specimen (urine) n (%)	direct collection	251 (62.7)	0.019*	138 (34.5)	0.093
	indirect collection (urinary catheter)	149 (37.2)		100 (25.0)	

Note: PHE – public hospital establishment; * – P less than 0.05.

Table 2
Prevalence of MDR and ESBL-producing uropathogens

Species	Number of isolates	Double-disk synergy test (DDST)		DDST + cloxacillin	Double disk test (DDT)		MDR strains No.
		number of ESBL-positive	number of ESBL-negative		number of ESBL-positive	number of ESBL-negative	
Escherichia coli	155	120	35	40	85	70	117
Klebsiella pneumoniae	39	33	6	13	33	6	28
Enterobacter cloacae	39	31	8	11	24	15	30
Raoultella ornithinolytica	25	19	6	3	10	15	17
Kluyvera spp.	22	17	5	0	7	15	14
Raoultella terrigena	12	10	2	6	12	0	8
Serratia liquefaciens	15	10	5	3	9	6	7
Proteus mirabilis	11	9	2	1	8	3	8
Serratia marcescens	11	8	3	2	7	4	7
Citrobacter freundii	9	8	1	2	6	3	8
Klebsiella oxytoca	7	5	2	3	6	1	2
Serratia odorifera	9	5	4	0	0	9	4
Citrobacter koseri	6	4	2	0	1	5	4
Enterobacter sakazakii	8	4	4	0	2	6	4
Other isolate	32	28	4	9	28	0	16
Total (%)	400 (100)	311 (77.7)	89 (22.3)	93 (23.2)	238 (59.5)	162 (40.5)	274 (68.5)

Note: other isolate – *Serratia fonticola* (n = 2), *Salmonella arizonae* (n = 2), *Salmonella choleraesuis* ssp. *arizonae* (n = 2), *Klebsiella pneumoniae* ssp. *ozanae* (n = 2), *Citrobacter braakii* (n = 2), *Cedecea lapagei* (n = 2), *Cedaceae deviser* (n = 1), *Enterobacter aerogenes* (n = 1), *Enterobacter amnigenus* (n = 1), *Escherichia fergusoni* (n = 2), *Morganella morganii* (n = 2), *Pantoea* ssp. 1 (n = 2), *Pantoea* ssp. 2 (n = 2), *Pantoea* ssp. 3 (n = 2), *Pantoea* ssp. 4 (n = 2), *Providencia alcafaciens* (n = 2), *Salmonella* spp. (n = 2), *Serratia plymuthyca* (n = 1).

As shown in Table 1, internal medicine, internal surgery, gynecology, dialysis and maternity grouped the majority of identified cases with 25.0%, 16.2%, 15.7%, 15.2%, 10.5% respectively. Only a reduced number from intensive care units 7 (1.7%) and orthopedics devices 1 (0.2%) were recorded. In addition, the public hospitals establishment of Tebessa and Bekkaria shared frequencies ranged from 25.2% to 30.5% of total confirmed cases.

According to the distribution of strains in terms of species, our finding shows that the predominance of *E. coli* and *Klebsiella pneumoniae* among isolated uropathogens, which were observed at 38.8% (155/400) and 9.8% (39/400), respectively. These results are in agreement with other studies which found that uropathogens are always predictable and *E. coli* and *Klebsiella pneumoniae* are the leading causes (Alasmary, 2021). Furthermore, the preponderance of these species could be due to the fact

as they possess a number of factors including adhesion, pilli, fimbrae, and P1 blood group genotype receptor, which contribute to the attachment of bacteria to the urothelium (Dash et al., 2018; Hantalo et al., 2020).

Antibiotics sensitivity pattern amongst Enterobacteriaceae isolates (Fig. 1) revealed high frequency of resistance to β-lactams ($P < 0.01$): amoxicillin (AMX) 89.3%, ticarcillin (TIC) 77.3%, ticarcillin + clavulanic acid (TTC) 72.5%, cephalexin (CL) 60.5% and cefixim (CFM) 45.0%. Regarding non-β-lactams antibiotics, resistance rates were high for ofloxacine (OF) 43.7% and cotrimoxazol (COT) 53.3%; significant for fosfomycine (FOS) 41.5%, and low for gentamicin (GM) 21.0%, amikacin (AK) 11.0%, nitrofurantoine (NIT) 8.0%. Only imipenem (IPM) and nitrofurantoin (NIT) were effective against more than 87.8% and 92.0% of the isolates respectively.

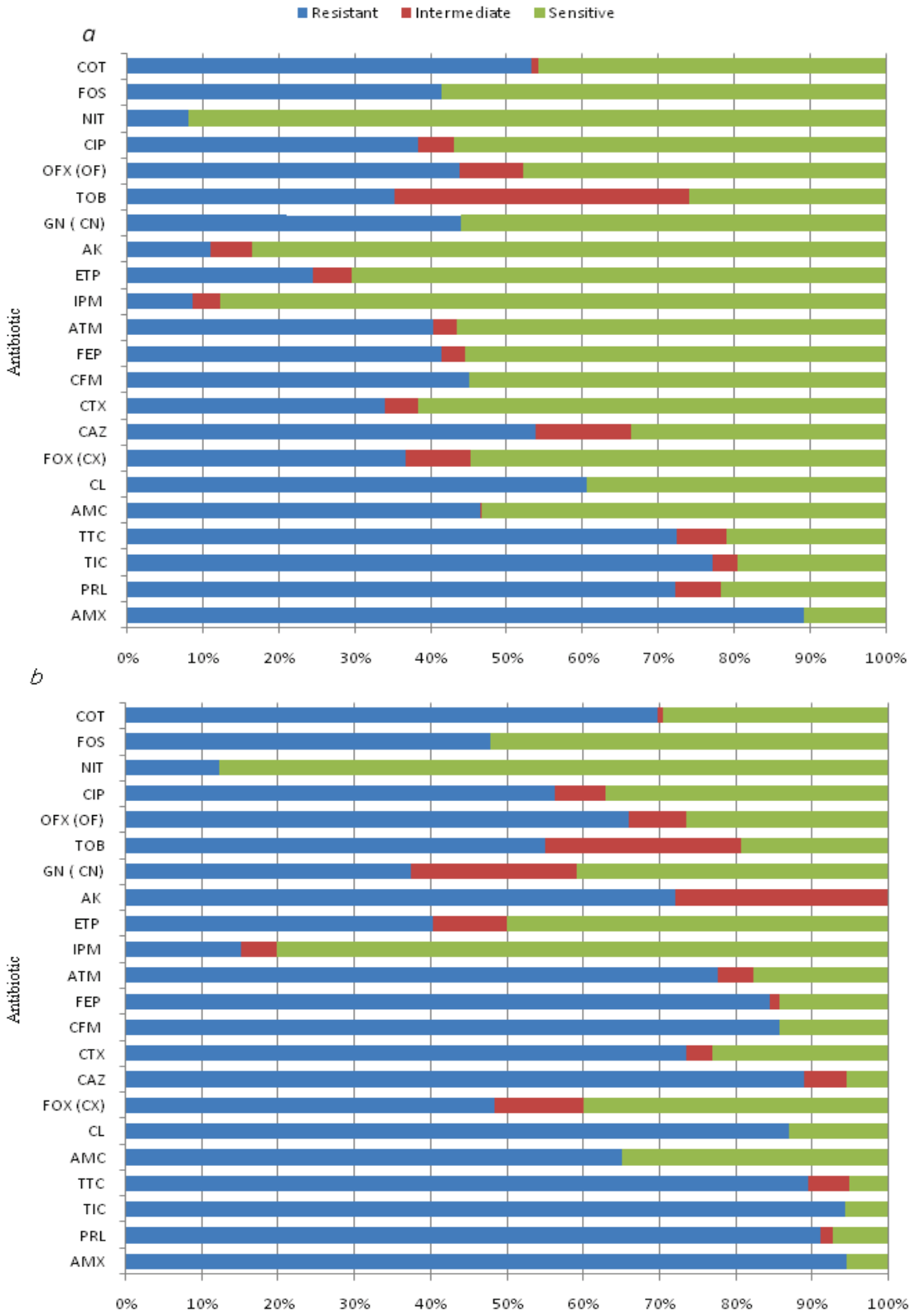

Fig. 1. Antimicrobial resistance pattern: of total Enterobacteriaceae isolates from patients with UTIs (n = 400) (*a*) and ESBL-positive strains (n = 238) (*b*): AMC: amoxicillin/ clavulanic acid, PIP: piperacillin, AMX: amoxicillin, TIC: ticarcillin, TTC: ticarcillin + clavulanic acid, CL: cephalexin, CTX: cefotaxime, CAZ: ceftazidime, ATM: aztreonam, IPM: imipenem, FOX: cefoxitin, AK: amikacin, GM: gentamicin, TOB: tobramycin, FOS: fosfomycin, FEP: cefepim, CIP: ciprofloxacin, NIT: nitrofurantoin, COT: cotrimoxazole, ETP: erthapenem, OFX: ofloxacin, CFM: cefexime

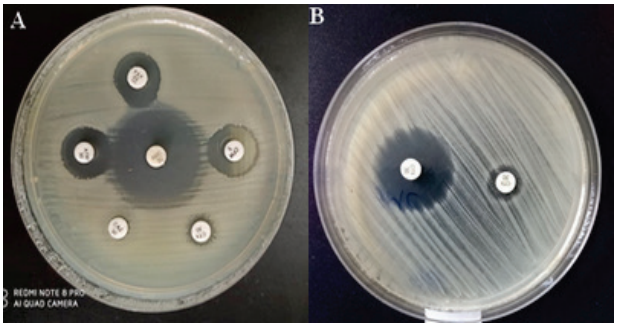

Fig. 2. Phenotypic detection of ESBLs producer by (*a*), double disk synergy test (DDST) (*b*), confirmed ESBLs producer by Double disk test (DDT)

Using the commonly used definition of multidrug resistance (MDR) as an organism being resistant to three or more classes of antibiotics, 68.5% (274/400) of the 400 isolates were identified as MDR, among which *E. coli*, *Enterobacter cloacae* and *K. pneumoniae* contributed to 29.2% (117/400), 7.5% (30/400) and 7.0% (28/400) of the observed MRD respectively (Fig. 3). The most effective antibiotics for MDR Gram negative bacilli were amikacin (97.1%), meropenem (96.3%), and imipenem (95.5%). Only 9.7% (39/400) of the Enterobacteriaceae were susceptible to all antibiotics tested in this study. The higher proportion of MDR recorded in this study was considered as alarming because it limits the treatment option of infections caused by uropathogenic bacteria. In recent years, Algeria has been considered among the developing countries that recorded high rates of MDRs, resulting in a reduction of therapeutic options and complicated first-line antibiotherapy (Muthupandian et al., 2018).

From the total 400 Enterobacteriaceae isolated, 311 (77.7%) were found to be suspicious for ESBLs production based on the result of the DDST method. Whereas the DDST methods on MH agar plates supplemented with cloxacillin showed that 93 of Enterobacteriaceae were ESBL producers. Using the double-disk test (DDT), we confirmed that 238 (59.5%) of the suspected isolates were able to produce ESBL. The distribution of uropathogenic ESBL-producing Enterobacteriaceae according to the species is shown in Figure 3. Amongst the 238 ESBL-E positive strains we found that 85 (35.7%) were represented by *E. coli*, 33 (13.9%) by *K. pneumoniae*, 24 (10.1%) by *E. cloacae*, and 10 (4.2%) by *Raoultella ornithinolytica*. The high percentage of ESBL-E obtained in this study is in agreement with several studies conducted in Algerian hospitals in which a high prevalence of ESβL-induced UTI was reported: University-Affiliated Hospital of Tlemcen (32.5%) (Zenati et al., 2019), and infectious disease service Boudjemaa Tourabi Public Hospital of Bechar (45.5%) (Benyagoub et al., 2021). In contrast to our study, a lower ESBL-E UTI prevalence (17.0%) was detected in Setif University Hospital (Nabti et al., 2019). Furthermore our finding is similar to that reported in many parts of the North African countries, such as in Morocco (Sbiti et al., 2017) in Libya (Abujnah et al., 2015) and in Tunisia (Alibi et al., 2015). In the current study, the high rate of ESBLs-producing uropathogens from UTI patients might be attributable to the local antibiotic prescribing policy, lack of antibiotic surveillance, misuse or overuse of antibiotics especially penicillin and third-generation cephalosporins (Mazzariol et al., 2017; Rania et al., 2019).

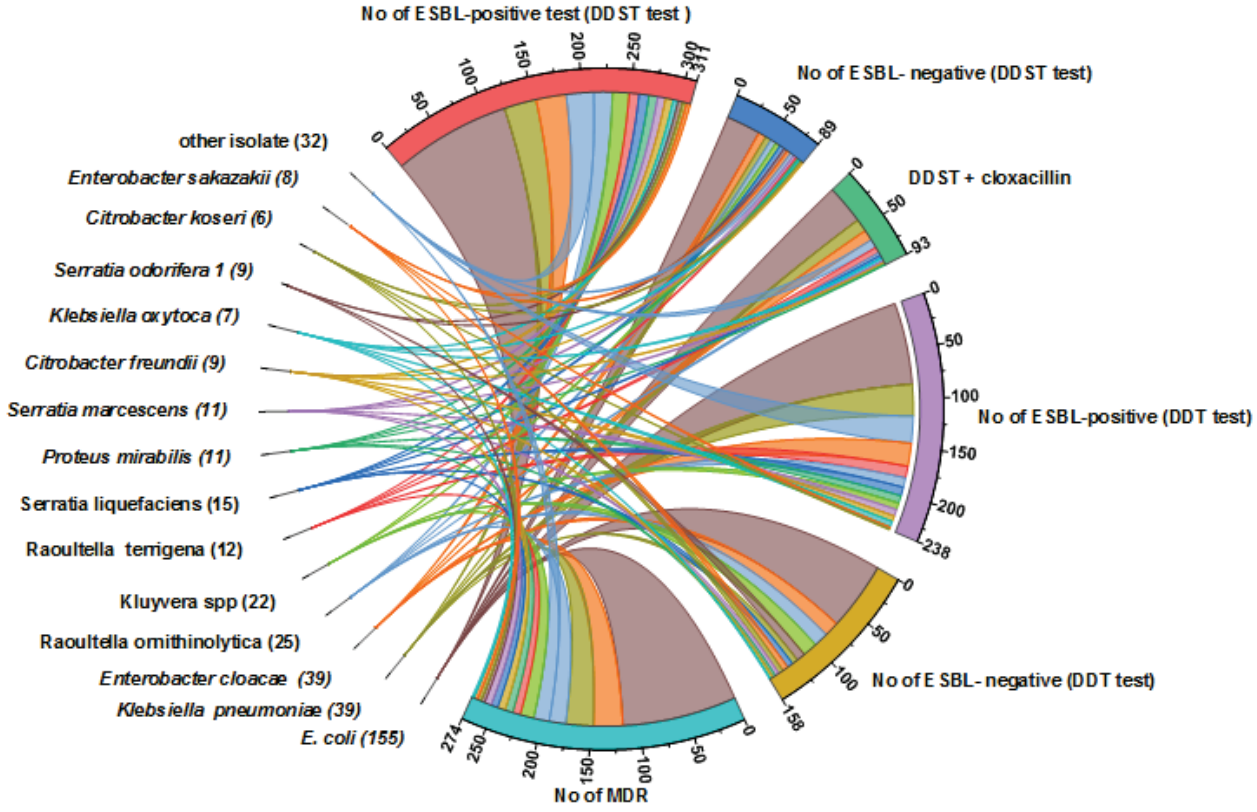

Fig. 3. Chord diagram showing the distribution of MDR and ESBL-producing uropathogens

The distribution of ESBL producers based on gender indicates that women had a higher prevalence rate of 57% than men, 43% (P = 0.006) from the total number of ESBL-E. There was no significant difference between the gender distributions and the source patients. In terms of age, our results showed that the most frequently ESBL-producing Enterobacteriaceae were related to the age adult patients of 20–39 years (124/400, 31.0%) compared with other age groups. Whereas the magnitude of ESBL-producing Enterobacteriaceae was different in the referral hospitals in Tebessa province. The magnitude was highest in EPH Tebessa and PHE Bekkaria (15.7%, 63/238) followed by PHE Bir El Ater (10.2%; 41/238) and PHE Ouinat (3.7%; 15/238), and lowest in PHE Morsott (0.7%; 3/238).

Recent data on ESBL-producing uropathogens in North African countries show an extremely worrying picture and this region could undoubtedly be one of the major epicenters of the global ESBL pandemic (Zenati et al., 2019; Hassuna et al., 2020). In a recent study conducted in

Egypt, Rania et al. (2019) reported the overall prevalence of 40% for ESBL producing organisms, which is almost similar to our data. Two other studies from Morocco have also reported high prevalence of ESBLs among UTIs Enterobacteriaceae (El bouamri et al., 2015; Halabi et al., 2021). Other investigators from the Middle East reported higher rates of ESBLs in Palestine (Tayh et al., 2019), Saudi Arabia (Somily et al., 2014) and Iraq (Al-Mayahie & Al Kuriashy, 2016), a higher frequency of ESBL-induced UTIs was found to be hospital acquired compared with community-acquired infections (53.3%, 67.0% and 65.5%, respectively).

According to the result of the antibiogram, the highest antibiotic resistance among ESBL-E was observed with amoxicillin, ticarcillin, third-generation cephalosporins (cefixime, cefotaxime and ceftazidime). Similar findings to this study were also reported in United Arab Emirates (Dash et al., 2018), in Libya (Mohammed et al., 2016), in Morocco (Natoubi et al., 2020). Many studies have reported an increasing resistance of ESBL-induced UTIs to most commonly used antimicrobials including third- and fourth-generation cephalosporins (Critchley et al., 2019; Ghravi et al., 2021).

The pattern of antimicrobial susceptibility among 238 ESBL-E showed high prevalence of resistance to various antimicrobial agents (Fig. 1), revealed high frequency of resistance to β-lactams ($P < 0.01$): amoxicillin (AMX) 94.5%, ticarcillin (TIC) 91.6%, ticarcillin + clavulanic acid (TTC) 89.5%, cephalexin (CL) 86.9%, ceftazidime (CAZ) 89.1%, cefepim (FEP) 84.4%, aztreonam (ATM) 77.7%, cefotaxime (CTX) 73.5% and amoxicillin/clavulanic acid (AMC) 65.2%. Regarding non-β-lactams antibiotics, resistance rates were high for ofloxacin (OFX) 65.9% and nalidixic acid (NA) 55.6%; significant for cotrimoxazole (COT) 69.7% and ciprofloxacin (CIP) 56.3%; moderate for tobramycin (TOB) 55.2%, fosfomycin (FOS) 47.9%, and low for gentamicin (GN) 37.3%, amikacin (AK) 18.5%, imipenem (IPM) 15.1% and nitrofurantoin (NIT) 12.2%.

Compared to our finding, multiple studies have clearly demonstrated that ESBL-producing uropathogens remained sensitive to imipenem and amikacin, and therefore these drugs have become the treatment of choice once the pathogens were identified and an antibiotic sensitivity report was generated in the laboratory, especially for treatment of the hospitalized patients (Raya et al., 2020; Alasmary, 2021). Halabi et al. (2021) confirmed that high resistance of ESBL-producing bacteria to large groups of antibiotics reduced considerably the therapeutic options and maintains a continuous pressure on the prescription of carbapenem antibiotics (Kharrat et al., 2018).

Conclusions

The emergence of Enterobacteriaceae producing ESBLs represents a real public health risk. In addition to the inactivity of all the therapeutic molecules of the beta-lactam class, these bacteria frequently exhibit multiple resistance mechanisms which can lead to a therapeutic impasse. Our study concluded that among phenotypically tested Enterobacteriaceae, 59.5% were ESBL producers. These organisms have become major etiological pathogens of community-acquired UTI in Algeria. These strains need to be efficiently detected in order to take appropriate preventive and therapeutic measures as quickly as possible with regard to the patients who harbour them. Their detection can sometimes be difficult but new means are gradually appearing to facilitate their detection in the microbiology laboratory.

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