ARTICLE ORIGINAL / ORIGINAL ARTICLE

LEBANESE OBSERVATORY OF PATHOGENIC AGENTS (LOPA-STUDY) A 2 YEAR-SURVEILLANCE PROSPECTIVE STUDY

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ABSTRACT • Background: An effective antimicrobial stewardship program, allowing the study of the evolution of the susceptibility profiles as well as the emergence of resistant strains, is essential at the national level. In Lebanon, a National Registry of the Epidemiology of bacterial pathogens find their susceptibility profiles is lacking. **Objectives:** Assessing the epidemiology of the main isolated bacteria as well as their distribution and susceptibility profiles. **Methods:** A multicenter study was performed in seven centers located in all districts in Lebanon over a period of 23 months (January 2015-November 2016) from ambulatory and hospitalized patients. Antibiotic susceptibility testing of the collected isolates was performed by the disc diffusion method (Kirby-Bauer), according to the joint recommendations of CASFM and EUCAST. **Results:** A total of 1026 strains of Gram negatives bacteria were identified and collected. Among them, *Escherichia coli* was the most commonly isolated (69%) followed by *Klebsiella pneumoniae* (11.4%), *Proteus mirabilis* and *Pseudomonas aeruginosa*. ESBL rates ranged from 4% (in *Proteus mirabilis*) to 34% in *E. coli* (34%) in parallel to lower susceptibility rates to third generation cephalosporins. All Gram-negative pathogens (expect for *A. baumanni*) still have high susceptibility rates to amikacin, carbanepems, and colistin. **Conclusions:** The results obtained from this study create a solid starting point for the observatory and give a comprehensive picture of the antibiotic resistance in the country.

Keywords : antibiotics; Lebanon; observatory; resistance

INTRODUCTION

Over the last 50 years, antibiotics have contributed to one of the greatest advances in medicine. Today, however, the emergence of pathogenic bacteria that have become resistant to antibiotics and their spread in the human population is a public health concern [1]. They seriously compromise the safety of patients with an increase in morbidity, hospitalization stay and costs as well as in mortality [2-5]. Hence, the United States Centers for Disease Control and Prevention (CDC) estimate that at least two million Americans become infected with antibiotic-resistant bacteria each year, with almost 23 000 people dying yearly as a direct result of these infections [6]. Moreover, a review on antimicrobial resistance published in 2014 have estimated the number of death to 10 million in 2050 [3].

Obtaining drugs without medical prescriptions can lead to the inappropriate use of ATBs [7], as over 50% of ATBs worldwide are purchased without a medical prescription [8], The determinants of self-medication with ATBs are well documented: their over-the-counter (OTC) availability [9], the cost of medical consultation, low satisfaction with medical practitioners [10] and misconceptions regarding the efficiency of ATBs [11]. All these factors contribute to the emergence and spread of multidrug resistant bacteria, especially those harboring extended-spectrum beta-lactamases (ESBLs). These strains can be found in hospital-acquired infections as well as in the community, which constitutes a real health care problem.

Recent findings suggest that, in hospital-acquired infections in particular, a significantly higher prevalence of strains, in particular, *Escherichia coli, K. pneumoniae* and *P. aeruginosa* are being resistant to antibiotics (multi-drug resistance profiles) [12].

Different factors could explain these results including local/regional medical practice with lack in resistance control stewardship strategies, lack of containment plans aiming at reducing the spread of resistant bacteria, and overuse or misuse of antibiotics. Hence, patients sometimes ask for medicines not intended for their medical condition and the health services tend to overprescribe them or are happy to provide them; not to mention the aggressive commercialization by industries and pharmaceutical companies that sometimes push doctors to prescribe newly marketed powerful antibiotics [2].

Gram-negative bacteria are largely responsible for community and hospital-acquired infections. Multidrug resistant strains are increasingly isolated in these settings, including carbapenemase-producing *Klebsiella*

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pneumoniae, *Acinetobacter and Pseudomonas aeruginosa* [13]. *Acinetobacter baumannii* has also become one of the most significant antibiotic-resistant bacteria causing hospital-acquired infections worldwide [14].

To overcome the problem of antimicrobial resistance, the implementation of an effective stewardship program with defined strategies and control policies, is essential at local and national levels. One of the strategies would be the study of the evolution of the susceptibility profiles as well as the emergence of resistant strains. Such information will provide the clinicians with specific data on patient's/local epidemiology allowing them to optimize the antibiotic treatment especially in empirical setting [15-17]. We conducted this study with the primary objective of assessing the main isolated bacteria and epidemiology of resistance, epidemiology and distribution of isolated bacteria as well as their susceptibility profiles. The aim of this prospective study was to achieve a National Registry of the Epidemiology of bacterial pathogens and their susceptibility to most commonly used antibiotics in Lebanon.

METHODOLOGY

Study design

The LOPA study is a microbiological in vitro multicenter study from seven centers located in all districts in Lebanon over a period of 23 months (01 January 2015-17 November 2016) from ambulatory and hospitalized patients. All Gram-negative specimens collected from pediatric patients, adults or elderly without any exclusion were included (non-duplicate isolates were not retained). All sources from all sites of monobacterial infection were to be included. These strains include bacteria from various sources: urine, bronchioalveolar lavage, sputum, blood culture, abscesses, wound culture (surgical and non-surgical), vaginal discharge, peritoneal fluid and stools. The target number of specimens over the study period was one thousand strains.

Data collected included the source of the specimen, the identified bacteria and the antibiotic used. Evaluated criteria included the distribution of isolated bacteria, the distribution of the sources of resistant bacteria, the percentage of resistance for each antibiotic and the percentage of multi-resistant bacteria.

Study protocol

The study protocol included several actions:

i. Collection of the bacterial strain and relevant susceptibility pattern from the participating centers;
ii. Purification, and deep-freezing of the strains;
iii. Determination of the types (identification) of isolated susceptible and resistant bacteria;
iv. Verification and realization of antibiotic susceptibility testing of the isolates according to CASFM - EUCAST recommendations;
v. Detecting and phenotypic confirmation of resistance mechanisms;
vi. Final study report including all isolated bacteria and susceptibility patterns.

Evaluation of antibiotic susceptibility

Antibiotic susceptibility testing (percentage of susceptible and intermediate values) of the collected isolates was performed by the disc diffusion method (Kirby-Bauer) [18], according to the joint recommendations of CASFM and EUCAST for 2016, a simple and routine phenotypic method for the detection of antibiotic resistance in bacteria, combined to the double disc synergy test when necessary (Appendix I and II). Susceptibility rates were calculated by including fully susceptible strains only. The production of ESBL was assumed when resistance to third generation cephalosporins (cefotaxime, ceftazidime) was seen. Confirmation was made by the synergy test with clavulanic acid: a positive synergy test implies the presence of an ESBL, while a negative one implies the presence of Amp C cephalosporinase. The percentage of ESBL producing bacteria was calculated by dividing the number of isolated ESBL strains by the number of total isolated strains. *Staphylococcus aureus* and *Streptococcus pneumoniae* were not included in the evaluation due to the poor number of isolates obtained. The antibiotics used for susceptibility testing are listed in Appendix III.

Quality control testing (QC) was performed using the EUCAST recommended Type Culture Collection (ATCC) QC strains.

Statistical analysis

Data was entered and analyzed, using the Statistical Package for Social Sciences (SPSS) version 22 software. Descriptive statistics were calculated for all study variables. This includes the counts and percentages for categorical variables.

RESULTS

One thousand and twenty-six (1026) strains of Gram negative rods were identified and collected. The collected bacteria belong to various species, as illustrated in the following Table I. *Escherichia coli* was the most commonly isolated bacteria in this study (69%). It was isolated in 78.72 % (640/813) of urinary tract infections, followed by *Klebsiella pneumoniae* (11.4%), *Proteus mirabilis* and *Pseudomonas aeruginosa* (3.7 and 3.8% respectively). In addition, among the collected Gram

TABLE I

SUMMARY OF THE COLLECTED BACTERIA IN THE LOPA STUDY

Species	Strains	
	Number	%
Escherichia coli	708	69
Klebsiella pneumoniae	114	11.2
Klebsiella oxytoca	47	4.6
Proteus mirabilis	24	2.3
Enterobacter cloacae	7	0.7
Citrobacter koseri	5	0.5
Serratia marcescens	4	0.4
Morganella morganii	5	0.5
Salmonella spp.	5	0.5
Pseudomonas aeruginosa	72	7
Pseudomonas spp.	14	1.4
Stenotrophomonas maltophilia	10	0.9
Acinetobacter baumannii	11	1.0
Total	**1026**	**100**

negative bacilli in this study, *E. coli* was the main agent in respiratory infections (31.6%), along with *Pseudomonas aeruginosa* (15.8%), and causes 53.8 % of body fluid infections.

The distribution of the strains per source (by number and by percentage) is shown in Tables II and III.

Susceptibility profiles of the isolated strains are presented in Table IV. In general, gram negative had a high susceptibility rates to imipenem, meropenem, amikacin but lower rates to fluoroquinolones and cephalosporins of third and fourth generation.

As for *P. aeruginosa,* a relatively good susceptibility to piperacillin/tazobactam and ciprofloxacin was noted (79% susceptibility rate each) as well as an excellent activity for meropenem (84%), amikacin (90%) and colistin (98%).

Finally, *A. baumannii* isolates showed very alarming susceptibility rates to various antibiotics even to carbapenems. The only remaining active drug was often colistin.

Rates of extended-spectrum beta-lactamase (ESBL) are also presented in Table IV. The highest percentage was noted among *Escherichia coli* (34%) and *Klebsiella pneumoniae* (25%), whereas the lowest rates occurred in *Proteus mirabilis* (4%).

DISCUSSION

Surveillance of antibiotic resistance and evaluation of the impact of control policies are at the core of successful antimicrobial stewardship programs [15-17]. The LOPA study was performed at a national level over a 2-year period of time aiming at achieving a national registry of the epidemiology of bacterial pathogens and their susceptibility profiles.

Our study showed that the most two prevalent bacteria isolated in urinary tract infections are *E. coli* and *K. pneu-*

TABLE II

Number of strains / Source	TOTAL	Urine	Broncho alveolar lavage	Sputum	Wound	Surgical wound	Blood	Abscess	Vaginal	Body fluid	Stools
Escherichia coli	708	640	6	11	14	-	9	7	7	14	-
Klebsiella pneumoniae	114	93	2	9	6	1	-	-	-	3	-
Proteus mirabilis	47	30	1	2	6	2	1	4	1	-	-
Enterobacter cloacae	24	9	2	2	6	-	3	-	2	-	-
Citrobacter koseri	7	4	-	-	1	-	-	-	1	1	-
Serratia marcescens	5	-	2	1	2	-	-	-	-	-	-
Morganella morgannii	4	3	-	-	-	-	-	-	-	1	-
Klebsiella oxytoca	5	-	-	4	1	-	-	-	-	-	-
Salmonella spp.	5	-	-	-	-	-	-	-	-	-	5
Pseudomonas aeruginosa	72	31	3	12	12	5	2	-	-	6	1
Pseudomonas spp.	14	1	3	5	3	1	1	-	-	-	-
Stenotrophomonas maltophilia	10	-	-	9	1	-	-	-	-	-	-
Acinetobacter baumannii	11	2	-	5	3	-	-	-	-	1	-
Total	**1026**	**813**	**19**	**60**	**55**	**9**	**16**	**11**	**11**	**26**	**6**

TABLE III

PERCENTAGE OF STRAINS PER SOURCE

Number of strains / Source	TOTAL	Urine	Alveolar lavage	Sputum	Wound	Surgical wound	Blood	Abscess	Vaginal	Body fluid	Stools
Escherichia coli	708	90.4	1.0	1.5	1.9	-	1.3	1.0	1.0	1.9	
Klebsiella pneumoniae	114	81.6	1.8	7.9	5.2	0.9	-	-	-	2.6	-
Proteus mirabilis	47	63.9	2.1	4.2	12.9	4.2	2.1	8.5	2.1	-	-
Enterobacter cloacae	24	37.5	8.4	8.4	24.7	-	12.6	-	8.4	-	-
Citrobacter koseri	7	57.1	-	-	14.3	-	-	-	14.3	14.3	-
Serratia marcescens	5	-	40	20	40	-	-	-	-	-	-
Morganella morgannii	4	75.0	-	-	-	X	-	-	-	25.0	-
Klebsiella oxytoca	5	-	-	80.0	20.0	-	-	-	-	-	-
Salmonella spp.	5	-	-	-	-	-	-	-	-	-	100
Pseudomonas aeruginosa	72	43.0	s	16.7	16.7	6.8	2.8	-	-	8.4	1.4
Pseudomonas spp.	14	7.2	21.4	35.6	21.4	7.2	7.2	-	-	-	-
Stenotrophomonas maltophilia	10	-	-	90.0	10.0	-		-	-	-	-
Acinetobacter baumannii	11	18.2	-	45.5	27.3	-	-	-	-	9.0	-

moniae. These results are in line with previous Lebanese studies [19,20], among them a very recent study evaluating the *Enterobacteriaceae* and non-fermentative Gram negative bacilli isolated in Lebanon over a five-year period, from 2011-2015 (Hajj *et al*. 2018; SMART study under revisions in *Future Microbiology*). As for the respiratory tract infections (RTI), *E. coli* was also the main isolated agent which is similar to another study performed in Lebanese centers showing that *Enterobacteriaceae* were the most commonly encountered pathogens whether in healthcare- or community-acquired infections [21]. Another study published in 2000 has shown that the Gram-negative bacilli accounted for the vast majority of all isolates isolated in hospital-acquired respiratory infections, the most commonly identified organisms being *Acinetobacter anitratus*, followed by *Pseudomonas aeruginosa* [22], Our study failed to identify *H. influenzae* among isolated pathogens, probably because the collection included more hospital- than community-acquired samples.

As for the susceptibility rates, *E. coli, K. pneumoniae,* as well as *E. cloacae* had lower susceptibility rates to third generation cephalosporins and this is probably due to the high rates of ESBL among these species. However, all Gram-negative pathogens (except for *A. baumanni*) still have high susceptibility rates to amikacin, carbanepems, and colistin as previously published in Lebanon [23-24].

In our study, the ESBL rates ranged from 4 to 25 and 34% (in *P. mirabilis, K. pneumoniae* and *E. coli* respectively). These ESBL rates are similar to those reported in a recent retrospective nationwide compiled data aiming at reporting the antimicrobial resistance in Lebanese hospitals (*E. coli* 32.3% and *Klebsiella spp*. 29.2%) [25]. However, they are higher than those published in UTI-associated *E. coli* isolates from Lebanese patients where prevalence of ESBLs was 16.8% in 2009 [26].

It is important to note that even if carbapenems maintain a good activity against almost all gram-negative bacteria, the emergence of carbapenem resistant strains is being reported. In our study, the resistance to imipenem and meropenem ranged from 1% to 4% among all gram-negative species. These results are in line with previously reported data in Lebanon that showed an overall prevalence of carbapenem-non-susceptible *Enterobacteriaceae* of 1.2% [27] and 1.6% [24]. The carbapenem resistance in *Enterobacteriaceae* in Lebanon appears to be modest particularly if we compare our results to the 4.2% reported by the Centers for Disease Control and Prevention (CDC) in 2011 [28]. It is noteworthy to mention that even if the numbers are still low, their rise should be expected if the antimicrobial resistance is not properly contained which limits the clinicians' treatment options. In *A. baumannii* associated infections, for example, the only remaining option is often colistin that is known to be associated with a large panel of side effects including severe nephrotoxicity that could be sometimes irreversible [129-31].

LIMITATIONS

Although the results obtained in this preliminary study were similar to the susceptibility percentages obtained in other studies cited above, additional analysis like the

TABLE IV ANTIBIOTIC SUSCEPTIBILITY OF GRAM NEGATIVE BACILLI ISOLATED IN THE LOPA STUDY (2015-2016)

Table 4.a. % susceptibility	Strains	**ESBL %**	AMP	AMC	TZP	KF	CXM	CFM	CTX	CAZ	AK	GN	NOR	PEF	CIP	TS	NI	FOT	AZT	IMP	MEM	ETP	CO	TGC
Escherichia coli	708	**34**	26	47	57	46	54	56	59	59	99	77	51	51	52	54	95	97	62	99	100	89	100	100
Klebsiella pneumoniae	114	**25**	0	56	62	64	68	68	68	68	99	79	68	68	70	65	45	92	71	99	99	95	100	96
Proteus mirabilis	47	**4**	53	85	94	81	89	94	96	96	100	81	87	87	87	57	0	85	0	96	100	100	0	98
Enterobacter cloacae	24	**17**	0	0	66	0	41	46	66	66	96	91	71	71	71	75	33	87	66	96	96	83	96	91
*Citrobacter koseri**	7	**42**	0	43	57	43	57	57	57	57	100	57	71	71	71	71	71	100	43	100	100	100	100	100
*Serratia marcescens**	5	**20**	0	0	80	0	0	80	80	80	100	100	60	60	60	100	0	80	80	100	100	100	0	100
*Morganella morgannii**	4	**0**	0	0	100	0	0	100	100	100	100	100	75	75	75	50	0	25	100	100	100	100	0	0
*Klebsiella oxytoca**	5	**0**	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
*Salmonella spp**	5	**0**	60	60	60	60	100	100	100	100	100	100	80	80	80	100	-	-	100	100	100	100	100	100

* Caution should be considered while interpreting the numbers and percentages due to small sample sizes

Table 4.b. % susceptibility	Strains	TZP	CAZ	CEF	IMP	MEM	AZT	AK	GN	TOB	LEV	CIP	TS	CO	FOT	TGC
Pseudomonas aeruginosa	72	79	76	43	78	84	58	90	85	92	69	79	0	98	75	-
Pseudomonas spp	14	85	85	64	78	85	42	78	78	78	50	64	64	85	71	0
S. maltophilia	10	0	20	0	0	0	30	60	70	70	70	70	100	60	-	60
Acinetobacter baumannii	11	9	0	0	18	18	0	27	18	18	27	27	54	90	-	54

Abbreviations (by order of appearance in table): AMP: ampicillin, **AMC**: amoxicillin + clavulanic acid, **TZP**: piperacillin + tazobactam, **KF**: cefalotin, **CXM**: cefuroxime, **CFM**: cefixime, **CTX**: cefotaxime, **CAZ**: ceftazidime, **FEP**: cefepime, **AZT**: aztreonam, **IMP**: imipenem, **MEM**: meropenem, **AK**: amikacin, **GN**: gentamicin, TOB: tobramycin, **FOT**: fosfomycin, **NOR**: norfloxacin, **PEF**: pefloxacin, **CIP**: ciprofloxacin, **TS**: cotrimoxazole, **NI**: nitrofurantoin, **CO**: colistin, **TGC**: tigecycline, **ETP**: ertapenem.

determination of the MIC (Minimal Inhibitory Concentration) and molecular genetic characterization (plasmid or chromosome encoded resistance) need to be performed in order to confirm the preliminary phenotypic results and define the type of ESBL and carbapenemases. Genetic methods such as PCR (Polymerase Chain Reaction) and sequencing are necessary to the description of the genes and a definite identification of the resistance determinants.

CONCLUSION

The susceptibility profiles obtained from this study create a solid starting point for the observatory and give a comprehensive picture of the antibiotic resistance in the country. The strains collected during this study will be stored and used for genetic for the coming years. They could also be used for the assessment of the susceptibility to new antibiotics or new treatment protocols and antibiotic associations. Sharing this data with the treating physicians would help them in the choice of antibiotics to use and would facilitate the fight against antibiotic resistance and multidrug-resistant bacteria.

Conflicts of interest

The authors declare no conflicts of interest.

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APPENDIX I

TABLE OF REFERENCE DIAMETERS (in mm) FOR THE ENTEROBACTERIA ACCORDING TO JOINT RECOMMENDATIONS OF CASFM AND EUCAST 2016

ANTIBIOTIC	RESISTANT if diameter is less than	SUSCEPTIBLE if diameter is equal or above
Amoxicillin	14	14
Amoxiciilin / Clavulanic acid	19	19
Piperacillin / Tazobactam	17	20
Cefalotin	14	14
Cefuroxime	18	18
Cefotaxime	17	20
Ceftazidime	19	22
Cefixime	17	17
Imipenem	16	22
Meropenem	16	22
Ertapenem	22	25
Amikacin	13	16
Gentamicin	14	17
Norfloxacin	19	22
Pefloxacin	19	22
Ciprofloxacin	19	22
Cotrimoxazole	13	16
Nitrofurantoin	11	11
Fosfomycin	13	16
Aztreonam	21	24
Tigecycline	15	18
Colistin	8	11

APPENDIX II

TABLE OF REFERENCE DIAMETERS (in mm) FOR *PSEUDOMONAS SPP*. ACCORDING TO JOINT RECOMMENDATIONS OF CASFM AND EUCAST 2016

ANTIBIOTIC	RESISTANT if diameter is less than	SUSCEPTIBLE if diameter is equal or above
Piperacillin /Tazobactam	17	20
Ceftazidime	19	22
Imipenem	16	22
Meropenem	16	22
Amikacin	13	16
Gentamicin	14	17
Tobramycin	14	17
Levoflaxin	19	22
Ciprofloxacin	19	22
Cotrimoxazole	13	16
Nitrofurantoin	11	11
Fosfomycin	13	16
Aztreonam	21	24
Colistin	8	11

APPENDIX III

The studied antibiotics are chosen according to CASFM-EUCAST recommendations:

– For Enterobacteria (including ESBL producing enterobacteria)

o Amoxicillin o Amoxicillin-Clavulanate o Piperacillin/Tazobactam o Cefalotine o Cefoxitin
o Cefuroxime o Cefotaxime o Ceftazidime o Cefepime o Cefixime o Imipenem o Meropenem
o Ertapenem o Amikacin o Gentamicin o Nalidixic acid o Ofloxacin o Ciprofloxacin o Cotrimoxazole
o Trimethoprim/Sulfamethoxazole o Nitrofurantoin o Fosfomycin o Aztreonam o Tigecycline o Colistin

– For *Pseudomonas aeruginosa*

o Piperacillin/Tazobactam o Ceftazidime o Cefepime o Imipenem o Meropenem o Tobramycin
o Amikacin o Levoflaxin o Ciprofloxacin o Aztreonam o Gentamicin o Colistin o Fosfomycin

– For *Acinetobacter baumannii*

o Piperacillin/Tazobactam o Ceftazidime o Cefepime o Imipenem o Gentamicin o Tobramycin
o Amikacin o Ciprofloxacin o Levofloxacin o Meropenem o Trimethoprim/Sulfamethoxazole o Tetracycline