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Original Article

Epidemiology and resistance profiles of Enterobacterales in a tertiary care hospital in Lebanon: a 4-year retrospective study

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Abstract

Introduction: Antimicrobial resistance (AMR) is a worldwide problem that threatens treatment effectiveness against the most serious bacterial infections. AMR in Enterobacterales is highly prevalent in Lebanon. However, recent reports regarding the distribution of Enterobacterales and related antimicrobial susceptibility are scarce.

Methodology: In this retrospective study at the Lebanese Hospital Geitaoui Medical Center in Lebanon, all data regarding culture specimens from urine, blood, sputum, deep tracheal aspirate, broncho-alveolar lavage, wounds, surgical sites, tissue, body fluids, and central venous catheter that were positive for at least one of the 4 bacterial isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Proteus mirabilis*) were collected. All susceptibility testing was performed according to the Clinical and Laboratory Standards Institute guidelines.

Results: A total of 4283 non-duplicate Enterobacterales were isolated during the study period (January 2017 to December 2020). Urine was the most common site of infection. *E. coli* was the most detected isolate as well as the leading pathogen in urine, wounds and surgical sites, and blood. Regarding antimicrobial susceptibility, the mean susceptibility to third generation cephalosporins was 55.53% and a mean extended-spectrum β-lactamases production of 31.2% was measured in *E. coli*. Mean carbapenem susceptibility was the lowest in *K. pneumoniae* and *E. cloacae*. The lowest mean susceptibility to fluoroquinolones was detected in *E. coli* isolates.

Conclusions: This study identified the predominance of *E. coli* among Enterobacterales in Lebanese patients, with the urinary tract being the most common site of infection and underlined the high rates of AMR in Enterobacterales in Lebanon.

Key words: Enterobacterales; AMR; ESBL; CRE; Lebanon.

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Introduction

The alarming increase of antibiotic resistance worldwide poses a catastrophic problem [1]. Indeed, antibiotic therapy is crucial for the treatment of bacterial infections [2], mainly in the intensive care units where serious and life-threatening infections like catheter-related bloodstream infections, ventilator associated pneumonia, urinary tract and surgical site related sepsis are commonly observed [3]. Members of the Enterobacterales are responsible for a large variety of intestinal and extraintestinal infections, where they are encountered most frequently in the urinary tract. They have also been detected in the blood, respiratory tract, peritoneal cavity, surgical sites, and wound isolates. These infections can be hospital or community acquired, affecting patients with or without preexisting illnesses [2,4,5]. Generally, antimicrobial resistance (AMR) can be due to antibiotic-inactivating enzymes such as β-lactamase or non-enzymatic mechanisms including efflux pump, outer membrane porins loss and antibiotic target modification [3]. For instance, in Gram-negative bacteria, including Enterobacterales, the most common mechanism of resistance is the production of β-lactamase. β-lactam drugs include penicillins, cephalosporins, carbapenems and monobactems [1,6,7]. β-lactamase can be either intrinsic or acquired [3]. However, β-lactamases can be divided into four large groups: extended-spectrum β-lactamases (ESBLs), AmpC-type cephalosporinases, carbapenemases and penicillinases [8]. ESBLs are the most common β-lactamases. They are able to hydrolyze all penicillins and cephalosporins, including third generation cephalosporins such as cefotaxime or ceftazidime [9]. Mobile genetic elements such as plasmids, carry β-lactamase encoding genes and can also transport genes responsible for resistance to

various antimicrobials such as tetracyclines, chloramphenicol, sulphonamides, aminoglycosides, trimethoprim, and fluoroquinolones [3,7]. In Lebanon, AMR is highly prevalent [10] and multiple studies on antimicrobial susceptibility patterns were done between 2011 and 2016 [11,12]. A high rate of ESBL infections (34%) has been reported in the last multi-centre study concerning antimicrobial susceptibility profiles in Lebanon in 2015-2016 [11]. Moreover, an increase in the prevalence of carbapenem resistant organisms including Enterobacterales has been observed in a 9-years single center study in Beirut [13]. A high rate of occurrence of methicillin resistant *Staphylococcus aureus* and multidrug resistant *Acinetobacter baumani* has been noticed [12]. In our present study, we analyzed the epidemiology of the most encountered Enterobacterales pathogens: *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterobacter cloacae* (*E. cloacae*) and *Proteus mirabilis* (*P. mirabilis*) isolated from different clinical specimens including urine, blood, respiratory specimens, wounds and surgical sites. Furthermore, we studied the profiles of antimicrobial susceptibility of these isolates in order to determine the different resistance profiles and their emergence.

Methodology

Study design and setting

This retrospective study was conducted at the Lebanese Hospital Geitaoui University Medical Center in Beirut, Lebanon (250 beds capacity) and included all isolates detected from urine, blood, respiratory specimens (sputum, deep tracheal aspirate and broncho-alveolar lavage), wound and surgical sites, and other sites of infection (including tissue, body fluids and central venous catheter) sent to the laboratory department between January 1st 2017 and December 31st 2020.

Data collection

An electronic search through the records of the laboratory information system data for all patients with culture specimens from urine, blood, sputum, deep tracheal aspirate, broncho-alveolar lavage, wounds, surgical sites, tissue, body fluids, and central venous catheter that were positive for at least one of the four bacterial isolates (*E. coli*, *K. pneumoniae*, *E. cloacae* and *P. mirabilis*) was conducted. The gender of each patient was noted. Moreover, infected sites were categorized into 5 groups: urine, blood, respiratory specimens (sputum, deep tracheal aspirate and broncho-alveolar lavage), wounds and surgical sites, and other sites of infection (including tissue, body fluids and central venous catheter). Antimicrobial susceptibility data was generated by the ADAGIO automated zone size reader for antimicrobial disk susceptibility tests (Bio-Rad Laboratories, Hercules, CA, USA). Culture duplicates from same patients were excluded.

Ethical consideration

The study was approved by the Research Ethics Committee of the Lebanese Hospital Geitaoui Medical Center. In accordance with the Declaration of Helsinki, all patients enrolled in this study provided written informed consent for both participation and publication of identifying information. Ethical clearance was taken as per the norms and in accordance with relevant guidelines and regulations of Lebanese Hospital Geitawi Medical Center. This study was done in a manner that ensured the confidentiality of patients.

Sample Size

A total of 4283 isolates were included in the study. Effectively, all cultures that were positive for at least one of the four bacterial isolates collected from the previously mentioned sites were included in the study.

Enterobacterales isolation and antimicrobial susceptibility testing

All Enterobacterales isolates included in the study were identified using biochemical gallery tests (API 20E; BioMérieux, Marcy-l'Etoile, France). Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using Kirby-Bauer disk diffusion method. All plates were read manually using a ruler followed by automatic reading using the ADAGIO system. The system is a combined imaging device and management software and automatically recognizes antibiotic disks on the agar and measures the surrounding inhibition zone diameters [14].

Phenotypic detection of ESBL and CRE

Susceptibility of Enterobacterales to cefotaxime and/or ceftazidime represented susceptibility to third-generation cephalosporins (3GC) and was a way to screen for ESBL production as per CLSI guidelines. CLSI recommends the use of any of the following antibiotic disks for ESBL screening in *E. coli*, *K. pneumoniae* and *P. mirabilis*: ceftazidime 30 µg, cefotaxime 30 µg, Aztreonam 30 µg [15]. However, CLSI confirmatory tests using ceftazidime or cefotaxime disks combined with clavulanate combination disks were not available. Another

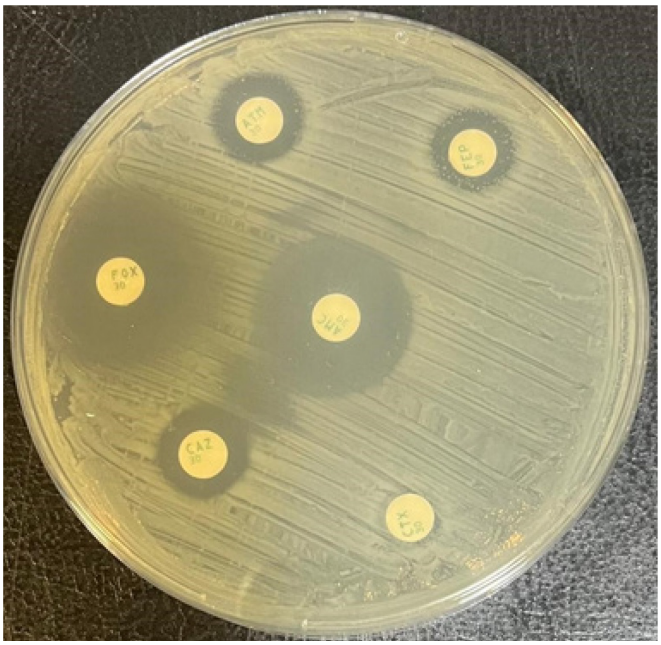
confirmatory test, the double disk synergy test (DDST), was used instead. 30 µg disks including cefotaxime, ceftazidime, aztreonam and cefepime were positioned at a distance of 30 mm from amoxicillin–clavulanate disk (20/10 µg). The test was considered positive when an enhancement of the inhibition zone of any of the antibiotic disks in front of the clavulanate containing disk was seen [16] (Figure 1). ESBL was not reported in *E. cloacae*.

Regarding carbapenems, imipenem and/or ertapenem were considered as markers of carbapenem susceptibility in Enterobacterales in this study. Enterobacterales that were resistant in vitro to any of the carbapenems were defined as carbapenem resistant Enterobacterales (CRE) as per Centers for Disease Control and Prevention (CDC) [17]. However, no confirmatory tests were available to confirm carbapenemase production.

Statistical analysis

Data was analyzed using Statistical Package for Social Science software (SPSS, Inc., version 26.0, Armonk, NY, USA). We analyzed bacterial isolates and sites of infection distribution among all cultures using the Chi-square test. Using the same test, bacterial distribution among different sites of infection was analyzed. Gender prevalence was presented as frequency and percentages. The level of significance was set at $p < 0.05$ for all statistical analyses. Antimicrobial susceptibility data was exported directly from ADAGIO automated zone size reader for antimicrobial disk susceptibility tests (Bio-Rad Laboratories, Hercules, CA, USA) and was presented as percentages.

Figure 1. Double disk synergy test (DDST) with synergistic effect in the Muller-Hinton plate. Synergy between amoxicillin - clavulanate disk and ceftazidime disk.



AMC: amoxicillin - clavulanate; ATM: aztreonam; CAZ: ceftazidime; CTX: cefotaxime; FEP: cefepime; FOX: cefoxitin.

Ethics approval and consent to participate

Ethical approval from the IRB committee at Lebanese Hospital Geitaoui was obtained (code: 2019-IRB-025).

Results

Prevalence of bacterial isolates

Among the 4283 isolates, *E. coli* accounted for the majority of positive cultures (72.89%), followed by *K. pneumoniae* (17.63%), *P. mirabilis* (6.14%) and *E.*

Figure 2. *E. coli*, *K. pneumoniae*, *E. cloacae* and *P. mirabilis* prevalence in 4238 specimens (p-value = < 0.05).

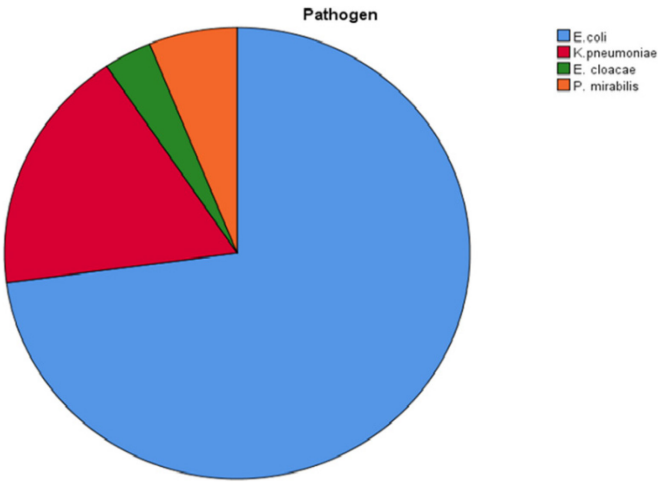
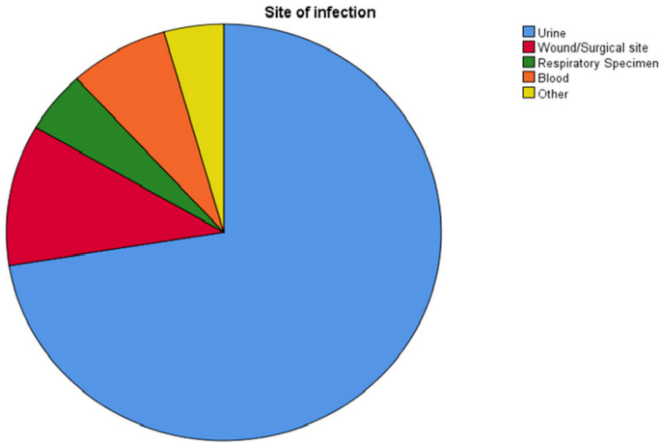


Figure 3. Distribution of infected specimens showing urine as the most common site of infection (p-value =< 0.05).



cloacae (3.34%) (Figure 2). Urine was significantly the most frequent site of infection. *E. coli* was the most common uropathogen (57.79%) followed by *K. pneumoniae* (10.09%) and *P. mirabilis* (3.69) (Figure 3). *E. cloacae* was isolated from 143 specimens, and was more prevalent in swab specimens from wound and surgical sites (41/143), in comparison to other sites of infection (urine 38/143, respiratory specimens 24/143 and blood 23/143). In wound and surgical site infections, *E. coli* was significantly the most frequently identified bacteria (259/469), followed by *K. pneumoniae* (99/469), *P. mirabilis* (70/469) and *E. cloacae* (41/469). *K. pneumoniae* was the most frequently identified pathogen in respiratory infections and was detected in 90 out of 205 respiratory specimens. *E. coli* was also responsible for a large number of pneumonias and isolated from 83 specimens. *E. cloacae* and *P. mirabilis* were isolated from 8 and 24 respiratory specimens respectively. Regarding bacteremia, *E. coli* contributed significantly to the majority of bloodstream infections (201/316) followed by *K. pneumoniae*, *E. cloacae* and *P. mirabilis* (Table 1).

Gender prevalence

E. coli, *K. pneumoniae* and *P. mirabilis* were most frequent in females (68.88%, 60% and 62.74% respectively). *E. cloacae* was slightly more common among the males (54.5%) (Table 2).

Antimicrobials susceptibility results

E. coli showed a mean susceptibility of 55.53% to third generation cephalosporins (3GC) and it was the lowest in 2020 (51.48%) in comparison to the previous years (highest in 2017: 57.57%). *K. pneumoniae* showed a mean susceptibility of 59.72% to the 3GCs which was lowest in 2019 (52.42%) and highest in 2017 (66.52%). *E. cloacae* and *P. mirabilis* showed higher susceptibility to 3GCs (87.42% and 92.53%) respectively.

Mean carbapenem susceptibility was the lowest in *K. pneumoniae* and *E. cloacae* (97.05% and 96.69%) and highest in *E. coli* and *P. mirabilis*. The mean prevalence of carbapenem resistant Enterobacterales (CRE) in *K. pneumoniae*, *E. cloacae*, *E. coli* and *P. mirabilis* was 2.95%, 3.71 %, 1% and 0.32% respectively. Regarding fluoroquinolones, *E. coli* had a mean of 56.86% susceptibility for ciprofloxacin, lower than *K. pneumoniae*, *E. cloacae* and *P. mirabilis* showing a susceptibility ranging from 75.85% and 91.98% (Tables 3 and 4).

Table 2. Gender distribution of bacterial pathogens.

	Male	Female
E. coli	973 (31.16%)	2149 (68.88%)
K. pneumoniae	302 (40.00%)	453 (60.00%)
E. cloacae	78 (54.50%)	65 (45.50%)
P. mirabilis	98 (37.26%)	165 (62.74%)

ESBL production

Mean ESBL production, confirmed by DDST, was 31.8% in *E. coli*, 30.4% in *K. pneumoniae* and 6.46% in *P. mirabilis*. In *E. coli*, ESBL production increased from 29.9% in 2017 up to 34.21% in 2020. ESBL producing *K. pneumoniae* were highest in 2018 (35.18%). Similar to *E. coli*, ESBL producing *P. mirabilis* increased from 3.92% in 2017 to 9.78% in 2020 (Table 5).

Discussion

A large variety of infections are caused by Enterobacterales that could be both, intestinal and extraintestinal. Among the latter, urinary tract is the most frequent site of infection followed by bloodstream, respiratory tract and surgical site infections [5]. This was shown in our study where urinary tract infections were the most common. The majority of urinary tract infections (UTIs) in this study were caused by *E. coli* (79.76%) followed by *K. pneumoniae* (13.92%) and *P. mirabilis* (5.09%). However, these uropathogens were reported as the most common causes of UTI's in other studies [18-20]. *E. cloacae* was most commonly found in wounds, surgical sites, urine, respiratory and blood specimens. However, *E. cloacae* has been described as a prevalent nosocomial pathogen in urinary tract infections, pneumonia and septicemia [21]. Generally, UTIs are more likely to occur in females.

Table 1. Distribution of infection sites among different bacterial isolates.

	E. coli	*K. pneumoniae*	*E. cloacae*	*P. mirabilis*	*p* value
Urine	2475	432	38	158	< 0.05
Wound/surgical site	259	99	41	70	< 0.05
Respiratory specimen	83	90	24	8	< 0.05
Blood	201	74	23	18	< 0.05
Others	104	60	17	9	< 0.05

A Chi-squared test was used to evaluate any significant difference between different bacterial isolates groups for each site of infection. The level of statistical significance was set at $p < 0.05$.

Table 3. Percentage of antibiotic susceptibility for *E. coli* and *K. pneumoniae* between 2017 and 2020.

	***E. coli* (% susceptible)**					***K. pneumoniae* (% susceptible)**				
	2017	**2018**	**2019**	**2020**	**Mean**	**2017**	**2018**	**2019**	**2020**	**Mean**
Amoxicillin / Clavulanic acid	52.47%	53.11%	53.81%	44.69%	51.02%	64.29%	53.09%	52.26%	60.82%	57.61%
Ampicillin	26.39%	25.26%	26.75%	26.21%	26.15%	-	-	-	-	-
Aztreonam	64.96%	61.01%	64.85%	58.98%	62.45%	68.75%	63.58%	60.97%	66.47%	64.94%
Cefixime	55.26%	53.24%	57.78%	51.38%	54.41%	66.07%	59.26%	52.26%	59.65%	59.31%
Cefuroxime	55.11%	48.11%	53.26%	51.03%	51.88%	66.00%	56.26%	49.29%	59.06%	57.65%
Piperacillin	30.13%	30.05%	29.57%	29.10%	29.72%	42.86%	36.42%	33.55%	39.18%	38.00%
Piperacillin / Tazobactam	79.88%	87.18%	91.22%	81.93%	85.05%	80.36%	77.78%	89.35%	77.19%	81.17%
Cefotaxime	55.77%	53.89%	57.89%	51.31%	54.71%	66.07%	59.26%	52.26%	59.88%	59.37%
Ceftazidime	59.37%	55.64%	58.73%	51.65%	56.35%	66.96%	60.49%	52.58%	60.23%	60.07%
Cefoxitin	85.74%	87.82%	87.37%	85.52%	86.61%	92.86%	94.44%	85.48%	83.63%	89.10%
Cefepime	62.97%	59.90%	65.20%	59.67%	61.93%	67.86%	62.96%	61.29%	67.84%	64.99%
Ciprofloxacin	55.62%	53.89%	59.04%	58.90%	56.86%	73.21%	77.78%	75.81%	76.61%	75.85%
Ertapenem	99.25%	99.61%	98.64%	98.34%	98.96%	95.54%	98.77%	97.42%	96.49%	97.05%
Imipenem	99.55%	99.61%	98.64%	98.34%	99.04%	95.54%	98.77%	97.42%	96.49%	97.05%
Amikacin	95.50%	97.67%	98.96%	99.17%	97.82%	93.75%	99.38%	98.06%	97.66%	97.21%
Gentamicin	82.68%	81.99%	83.49%	82.90%	82.77%	77.48%	83.95%	80.00%	87.13%	82.14%
Tetracycline	46.03%	52.85%	51.20%	55.59%	51.42%	64.29%	67.28%	63.87%	65.50%	65.23%
Tigecycline	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	99.35%	100.00%	99.84%
Trimethoprim-sulfamethoxazole	53.38%	52.72%	49.95%	55.03%	52.77%	57.14%	55.56%	52.26%	59.65%	56.15%

Table 4. Percentage of antibiotic susceptibility for *E. cloacae* and *P. mirabilis* between 2017 and 2020.

	***E. cloacae* (% susceptible)**					***P. mirabilis* (% susceptible)**				
	2017	**2018**	**2019**	**2020**	**Mean**	**2017**	**2018**	**2019**	**2020**	**Mean**
Amoxicillin / Clavulanic acid	0.00%	0.00%	0.00%	0.00%	0.00%	92.16%	90.38%	82.28%	74.07%	84.72%
Ampicillin	0.00%	0.00%	0.00%	0.00%	0.00%	35.29%	51.92%	45.57%	34.57%	41.84%
Aztreonam	93.75%	86.67%	85.71%	89.74%	88.97%	98.04%	98.08%	96.20%	90.12%	95.61%
Cefixime	93.55%	83.33%	85.71%	87.18%	87.44%	96.08%	94.23%	88.61%	87.65%	91.64%
Cefuroxime	0.00%	0.00%	0.00%	0.00%	0.00%	90.20%	94.23%	86.08%	83.95%	88.61%
Piperacillin	87.50%	73.33%	78.57%	82.05%	80.36%	76.47%	82.69%	73.42%	62.96%	73.89%
Piperacillin / Tazobactam	93.75%	83.33%	85.71%	87.18%	87.49%	100.00%	100.00%	100.00%	96.30%	99.07%
Cefotaxime	93.75%	83.33%	85.71%	87.18%	87.49%	96.08%	94.23%	88.61%	87.65%	91.64%
Ceftazidime	93.75%	83.33%	85.71%	87.18%	87.49%	98.04%	98.08%	89.87%	87.65%	93.41%
Cefoxitin	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	100.00%	93.67%	97.53%	97.80%
Cefepime	93.75%	93.33%	90.48%	92.31%	92.47%	98.04%	96.15%	93.67%	90.12%	94.50%
Ciprofloxacin	96.88%	83.33%	92.86%	94.87%	91.98%	78.43%	84.62%	75.95%	80.25%	79.81%
Ertapenem	100.00%	96.67%	95.24%	94.87%	96.69%	100.00%	100.00%	98.73%	100.00%	99.68%
Imipenem	100.00%	96.67%	95.24%	94.87%	96.69%	100.00%	100.00%	98.73%	100.00%	99.68%
Amikacin	96.88%	100.00%	100.00%	100.00%	99.22%	98.04%	98.08%	100.00%	100.00%	99.03%
Gentamicin	96.88%	90.00%	95.24%	97.44%	94.89%	70.59%	76.92%	69.62%	74.07%	72.80%
Tetracycline	62.50%	63.33%	80.95%	84.62%	72.85%	0.00%	0.00%	0.00%	0.00%	0.00%
Tigecycline	100.00%	100.00%	100.00%	100.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Trimethoprim-sulfamethoxazole	81.25%	70.00%	83.33%	82.05%	79.16%	47.06%	44.23%	44.30%	33.33%	42.23%

Table 5. Rate of extended-spectrum β-lactamases (ESBL) production, confirmed by the double disk synergy test (DDST), in the three studied organisms (*E. coli*, *K. pneumoniae* and *P. mirabilis*).

	2017	**2018**	**2019**	**2020**	**Total**
E. coli	200/667 (29.9%)	262/772 (33.9%)	282/958 (29.4%)	248/725 (34.2%)	992/3122 (31.8%)
K. pneumoniae	30/112 (26.78%)	57/162 (35.18%)	103/310 (33.22%)	40/171 (23.4%)	230/755 (30.4%)
P. mirabilis	2/51 (3.92%)	3/52 (5.76%)	4/79 (5.06%)	8/81 (9.87%)	17/263 (6.46%)

Consistent with our findings, UTIs are the most common infections and are more likely to occur in females owing to physical and anatomical factors, specifically the short distance between the genital and urinary systems [18]. As for antimicrobial susceptibility, the last year (2020) showed the lowest susceptibility for the 3GCs in comparison to the previous years (2017 to 2019) and to other studies conducted between 2011 and 2017 [11,12,18]. Ceftazidime susceptibility in *E. coli* decreased progressively from 75.6% in 2011 to 69.1% and 62% in 2013 and 2015 respectively [11,12]. Cefotaxime and ceftriaxone showed a similar decrease [11,12]. Our data showed a higher and progressive decrease in 3GC's susceptibility in the following years. Regarding the ESBL production, an average production in *E. coli* and *K. pneumoniae* of 32.3% and 29.2% in Lebanon between 2011 and 2013 was reported [12]. An increase in ESBL production in Enterobacterales was noticed in the 2015/2016 multi-centric study reaching 43% [11]. However, ESBL is the major mechanism of resistance to extended spectrum cephalosporins [8]. Our results showed a mean ESBL production in *E. coli* and *K. pneumoniae* similar to the 2015/2016 data and 2011-2013 study [11,12]. However, our data showed a progressively increasing pattern of ESBL in *E. coli*. Moreover, multiple studies were conducted in Lebanon regarding ESBL enzymes [22,23], identifying CTX-M enzymes as the most common type. An epidemiological issue regarding these enzymes is that they are mainly found in healthy *E. coli* colonized subjects, thus forming a community reservoir and leading to increased community and hospital acquired ESBL infections [3]. In Lebanon, a high ESBL producing Enterobacterales carriage (24.8 %) among healthy children was demonstrated in a study conducted by Hijazi *et al*. [9]. Similarly, a high carriage of ESBL *E. coli* (52.9%) among food workers in North Lebanon was shown in a recent report [24]. Moreover, ESBL pathogens were demonstrated as the major cause of multidrug resistant bloodstream infections in patients with hematological malignancies in a recent study [25]. One major risk factor for ESBL acquisition is antibiotic abuse [7] which was shown to be highly prevalent in Lebanon [26].

Regarding carbapenems, the average susceptibility for *E. coli*, *K. pneumoniae*, *E. cloacae* and *P. mirabilis* was 99%, 97.05%, 96.69% and 99.68%. However, an average carbapenem susceptibility of 99.3% and 98% was reported between 2011 and 2013 [12]. Our results correspond with previous studies where a mean prevalence of 1.2% of CRE was reported by Hammoudi *et al.* [27]. Furthermore, carbapenem resistance ranging from 1 to 5% for *E. coli* and 1 to 10% for *Klebsiella* spp was reported in 11 hospitals during 2015 and 2016 which are consistent with our data. The emergence of CRE in Lebanon is mainly attributed to OXA-48 carbapenemase production, which was first discovered in 2008 [11,28].

In comparison to the 2015-2016 study conducted by Moghnieh *et al*., the susceptibility of *E. coli* and *K. pneumoniae* to fluoroquinolones were similar. Regarding amikacin, gentamicin, tigecycline and trimethoprim-sulfamethoxazole (TMP/STX) susceptibility, our data showed no decrease in susceptibility for either *E. coli* or *K. pneumoniae* [11].

As for *P. mirabilis*, a progressive decrease in susceptibility to amoxicillin/clavulanic acid, second-, third-, fourth-generation cephalosporins, ciprofloxacin and TMP/SMX was observed. Moreover, there was resistance to non-beta-lactam antibiotics among ESBL producers. This includes fluoroquinolones, trimethoprim, sulfonamides, aminoglycosides and other drugs [9]. Furthermore, an increased ESBL production was shown in our results reaching 9.88% in 2020. ESBL producing *P. mirabilis* isolates have been reported in UTI's in the smart 2010-2014 analysis, and ranged between 6.1% and 11.4% of total isolated *P. mirabilis* in urine [29]. ESBL producing *P. mirabilis* isolates were identified in other sites also such as bacteremia, respiratory, wound and ulcers infections [30,31].

In the case of *E. cloacae*, chromosomal *AmpC* genes encode AmpC-type cephalosporinase, thus conferring intrinsic resistance to penicillins, first- and second-generation cephalosporins and cephamycins [21]. Therefore, all isolates were resistant to the previously mentioned drugs. An increasing pattern of resistance was seen mainly in the last 3 years, reaching 12.51% for third generation cephalosporins. Furthermore, an extended resistance to third-generation cephalosporins and aztreonam can be seen in AmpC hyperproduction, which results from chromosomal mutations. In addition, an increased prevalence of ESBL production is reported in *E. cloacae* pathogens [21]. However, β-lactamase inhibitors, clavulanic acid in particular, have a weak activity against AmpC β-lactamase [32] and a synergistic effect between clavulanate and cephalosporins disks is problematic. Other inhibitors can be used to identify AmpC β-lactamase, such as cloxacillin and boronic acid and differentiate it from ESBL β-lactamase [33]. While carbapenem susceptibility was 100% in 2017, a decreased susceptibility was observed with the lowest

seen in 2020 (94.69%). This finding matches with the increasing emergence of CRE *E. cloacae* pathogen in reports [34, 35]. Similarly, these pathogens were reported in United states as the second most common CRE [21].

The increase in antibiotic resistance in Lebanon could be attributed to various factors. Surface water contamination with multidrug resistant bacteria, including ESBL and carbapenemase producers, was recently reported [36]. In addition, increased antibiotic usage is the main contributor to AMR. In particular, a correlation between ESBL production and antibiotic usage has been reported [37]. Thus, the increased consumption of antibiotics among the Lebanese community, including the broad spectrum antibiotics have a major role in AMR [38]. Moreover, a poor level of antimicrobial stewardship program (ASP) where there is absence of clear national and in-hospital guidelines, shortage of infectious disease physicians, as well as lack of regular education programs for medical staff and minimal support from Ministry of public health were reported by Lebanese physicians [39].

We acknowledge that our study had several limitations, one of which is related to the data being collected from one medical center and the lack of confirmatory tests for carbapenemase production. However, there is a scarcity of updated data regarding antimicrobial susceptibility in Lebanon between 2017 and 2020 which is the strength of the study.

Conclusions

This study identified the predominance of *E. coli* among Enterobacterales in Lebanese patients, with urinary tract being the most common site of infection. It also underlined the increase of resistance to third generation cephalosporins and the high rate of ESBL producing organisms during the study period in comparison to the previous years. A rise of CRE and multidrug resistant bacteria during the study period is a pattern of concern. These results highlight a major problem in the healthcare system in Lebanon regarding the high prevalence of inappropriate antibiotic prescriptions and poor antimicrobial stewardship program (ASP) program in Lebanese hospitals. The need for a vigorous national AMR surveillance system and accurate ASP are urgently needed.

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