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A retrospective study of antibiotic resistance patterns of bacterial pathogens isolated from patients in two Lebanese hospitals for two consecutive years (2018 and 2019)

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Abstract:

Background: Misuse of antibiotics is the leading factor promoting emergence of bacterial resistance, a situation that has become a serious public health challenge. Among the leading bacteria that have developed resistance to antibiotics are *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which have caused infections in patients, resulting in considerable mortality. The objective of this retrospective study was to assess antibiotic resistance rates of bacterial pathogens isolated from clinical specimens in two Lebanese hospitals between the years 2018 and 2019.
Methodology: Bacteria isolated from routine clinical specimens collected from hospitalized patients in two hospitals, Haroun and Bekaa, in Lebanon for 2018 and 2019, were analyzed. Bacteria isolation and identification were carried out at the laboratory of each hospital using conventional microbiological methods. Antimicrobial susceptibility testings (AST) of each bacterial isolate to antibiotics were performed by the disc diffusion test and interpreted using EUCAST, CLSI or WHO/AST guidelines. Comparisons of the mean resistance rates of each isolate to individual antibiotics by year of isolation were done using the Z-test and $p< 0.05$ was considered statistically significant.
Results: There were a total of 1698 bacteria isolates recovered from hospitalized patients in the two hospitals for 2018 and 2019, of which 87.5% were Gram-negative and 12.5% were Gram-positive bacteria. The most frequent among the Gram-negative isolates was *E. coli* (66.1%) followed by *P. aeruginosa* (13.3%), *K. pneumoniae* (7.7%), *Proteus mirabilis* (6.7%) and *Enterobacter* spp (6.3%), while coagulase positive staphylococci CoPS (68.4%) and *E. faecalis* (31.6%) were the two Gram positive isolates. Of the Gram-negative isolates over the two-year period, 72.2% of *E. coli* and 76.3% of *K. pneumoniae* were resistant to ceftazidime, 93% of *P. mirabilis* to colistin, and 98% of *Enterobacter* to cefoxitin, but low resistance rates were demonstrated by *E. coli* to imipenem (1%), *K. pneumoniae* to tigecycline and amikacin (0.9%), *P. mirabilis* to imipinem (2%), and *Enterobacter* to amikacin, ertapenem and tigecycline (3%). Resistance of *P. aeruginosa* varied between 2% to colistin and 24% to levofloxacin. For the Gram-positive bacteria, 79.1% of *E. faecalis* were resistant to erythromycin while 70% of CoPS were resistant to cefoxitin, but no isolate was resistant (0%) to linezolid, and only 1% to teicoplanin. Except for *Enterobacter* spp that showed significant increase in resistance rates (by 250%) to piperacillin/tazobactam in 2019 over 2018, resistance rates of other Gram-negative isolates significantly decreased in 2019 compared to 2018 ($p<0.05$). For the Gram-positive isolates, resistance rates to many antibiotics tested significantly increased (by a factor of 36.5 - 2569%) in 2019 compared to 2018 among *E. faecalis* isolates in contrast to the rates for CoPS which significantly decreased by 16.7 - 65.7%, except for penicillin G which increased by a factor of 123%.
Conclusion: Overuse and misuse of antibiotics, which is possible because of the easy access of the populace to these drugs, is a leading factor contributing to the high antibiotic resistance rates in this study. There is need to promote awareness of antimicrobial resistance in Lebanon among students especially in non-health related majors and enactment of govermental policy that will limit access to antibiotics.

Keywords: antibiotic resistance; changing pattern; hospitalized patients; retrospective

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Une étude rétrospective des profils de résistance aux antibiotiques de pathogènes bactériens isolés de patients dans deux hôpitaux libanais pendant deux années consécutives (2018 et 2019)

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Abstrait:

Contexte: La mauvaise utilisation des antibiotiques est le principal facteur favorisant l'émergence de la résistance bactérienne, une situation qui est devenue un sérieux défi de santé publique. Parmi les principales bactéries qui ont développé une résistance aux antibiotiques figurent *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* et *Pseudomonas aeruginosa*, qui ont provoqué des infections chez les patients, entraînant une mortalité considérable. L'objectif de cette étude rétrospective est d'évaluer les taux de résistance aux antibiotiques des pathogènes bactériens isolés à partir d'échantillons cliniques dans deux hôpitaux Libanais entre les années 2018 et 2019.
Méthodologie: Les isolats bactériens prélevés sur des patients hospitalisés dans deux hôpitaux, Haroun et Bekaa, au Liban pour 2018 et 2019, ont été analysés. L'isolement et l'identification des bactéries ont été réalisés au laboratoire de chaque hôpital en utilisant des méthodes microbiologiques conventionnelles. Les tests de sensibilité aux antimicrobiens (AST) de chaque isolat bactérien aux antibiotiques ont été réalisés par le test de diffusion sur disque et interprétés selon les directives EUCAST, CLSI ou WHO/AST. Des comparaisons des taux moyens de résistance de chaque isolat à des antibiotiques individuels par année d'isolement ont été effectuées à l'aide du test Z et $p<0,05$ a été considéré comme statistiquement significatif.
Résultats: Il y a eu un total de 1698 isolats de bactéries récupérés de patients hospitalisés dans les deux hôpitaux durant 2018 et 2019, dont 87,5% étaient à Gram négatif et 12,5% étaient des bactéries à Gram positif. Les isolats à Gram négatif les plus fréquents étaient *E. coli* (66,1%), suivis de *P. aeruginosa* (13,3%), *K. pneumoniae* (7,7%), *Proteus mirabilis* (6,7%) et *Enterobacter* spp (6,3%), tandis que les staphylocoques à coagulase positive CoPS (68,4%) et *E. faecalis* (31,6%) étaient les deux isolats Gram positifs. Parmi les isolats à Gram négatif sur la période de deux ans, 72,2% d'*E. coli* et 76,3% de *K. pneumoniae* étaient résistants à la ceftazidime, 93% de *P. mirabilis* à la colistine et 98% d'*Enterobacter* à la céfoxitine, mais faible les taux de résistance ont été démontrés par *E. coli* à l'imipénem (1%), *K. pneumoniae* à la tigécycline et à l'amikacine (0,9%), *P. mirabilis* à l'imipinem (2%) et *Enterobacter* à l'amikacine, à l'ertapénem et à la tigécycline (3%). La résistance de *P. aeruginosa* variait entre 2% à la colistine et 24% à la lévofloxacine. Pour les bactéries Gram positif, 79,1% des *E. faecalis* étaient résistantes à l'érythromycine tandis que 70% des CoPS étaient résistantes au céfoxitin, mais aucun isolat n'était résistant (0%) au linézolide et seulement 1% à la teicoplanine. À l'exception d'*Enterobacter* spp qui ont montré une augmentation significative des taux de résistance (de 250%) à la pipéracilline/tazobactam en 2019 par rapport à 2018, les taux de résistance des autres isolats à Gram négatif ont considérablement diminué en 2019 par rapport à 2018 ($p<0,05$). Pour les isolats Gram-positifs, les taux de résistance à de nombreux antibiotiques testés ont augmenté de manière significative (d'un facteur de 36,5 à 2569%) en 2019 par rapport à 2018 parmi les isolats d'*E. faecalis* contrairement aux taux de CoPS qui ont significativement diminué de 16,7 à 65,7%, à l'exception de la pénicilline G qui a augmenté d'un facteur de 123%.
Conclusion: la surutilisation et la mauvaise utilisation des antibiotiques, ce qui est possible en raison de l'accès facile de la population à ces médicaments, est l'un des principaux facteurs contribuant aux taux élevés de résistance aux antibiotiques dans cette étude. Il est nécessaire de promouvoir la sensibilisation à la résistance aux antimicrobiens au Liban parmi les étudiants, en particulier dans les spécialisations non liées à la santé, et la promulgation d'une politique gouvernementale qui limitera l'accès non contrôlé aux antibiotiques.

Mots clés: résistance aux antibiotiques; changement de modèle; patients hospitalisés; rétrospective

Introduction:

Antimicrobial resistance (AMR) occurs when the drug loses its ability to effectively inhibit bacterial growth (1). In the developing countries, almost all antibiotics are available and can be purchased without medical prescription, which is one of the main factor underlying emergence of antimicrobial resistance (1). AMR is creating a serious global public health threat (2). In 2017, 12 bacterial species were identified by the World Health Organisation (WHO) to represent a threat to human health with majority being Gram negative bacteria (GNB), such as *Acinetobacter, Pseudomonas aeruginosa,* Enterobacteriaceae*, Helicobacter pylori, Salmonella* spp.*, Neisseria gonorrhoeae* and *Shigella* spp (3). The Enterobacteriaceae are

now globally reported to be resistant to carbapenems, third generation cephalosporins, and colistin (3).

Recently, the European Center for Disease Prevention and Control (ECDC) reported that GNBs are responsible for more than 500,000 infections and more than 24,600 deaths in Europe in just one year (4). The WHO press release highlights the real threat of GNBs which have developed remarkable mechanisms enabling them to resist antibiotic actions (5). In the United States and European countries, the number of deaths due to antibiotic resistance in *Staphylococcus aureus, Escherichia coli, Enterococcus faecium, Klebsiella pneumoniae* and *P. aeruginosa* was estimated to be 50,000 yearly (6). Laws et al., (7) reported that between 2011 and 2014, the percentage of *K. pneumoniae* and *E. coli* infections resistant to fluoroquinolones, third-generation cephalosporins or aminoglycosides, as well as combined resistance to all three antibiotic groups, significantly increased in Europe.

The WHO global report on antimicrobial resistance indicates that resistance of common bacteria has reached alarming levels in many African countries, as over 50% of *E. coli* and *Klebseilla* isolates were reported to be resistant to the third generation cephalosporins and carbapenems (5). Mouiche et al., (8) also reported in Cameroon in 2019 high levels of resistance of *E. coli, Klebsiella* sp, *S. aureus, P. aeroginosa, Enterobacter* spp*,* and *Proteus* spp to tetracycline, amoxicillin, nalidixic acid, ampicillin and trimethoprim but low levels of resistance to gentamicin, ceftriaxone and ciprofloxacin. Ahmed et al., (9) reported in 2019 that the prevalence of *E. coli* resistance to most antibiotics such as amoxicillin, amoxicillin/clavulanic acid, ampicillin and ciprofloxacin was very high in Bangladesh. Resistance patterns of microbes vary from country to country, large hospital to small hospital, and from hospital to the community (10). Among these increasingly antimicrobial resistance infections are methicillin resistant *S. aureus* (MRSA), which have become prevalent in many countries, including the USA, European countries, South America and Asia (11). Another growing threat worldwide is *E. coli* and *K. pneumoniae* harboring extended-spectrum beta lactamases (ESBL) (12).

In the past few years, in the Middle East, the frequency of drug-resistant bacteria isolates has been increasing in different hospitals as confirmed by the WHO (13). Over 700,000 deaths are reported yearly due to AMR, and in the absence of appropriate control and preventive measures, AMR is projected to become one of the main causes of death among hospitalized and non-hospitalized patients in the developed countries (14). In the Mediterranean countries, many studies have reported emergence of bacterial resistance mechanisms such as ESBL, AmpC and carbapenemases in Gram negative bacteria, methicillin and vancomycin resistance in Gram positive organisms, and penicillin resistance in *Streptococcus pneumoniae* (15).

In Lebanon, as in other developing countries, AMR is responsible for significant morbidity and mortality in different hospitals (16). The most important factor leading to the emergence of AMR is the uncontrolled or inappropriate use (misuse and overuse) of antimicrobial drugs (17). This is mainly due to incorrect diagnosis and missuse of antimicrobials due either to an inappropriate prescription or poor compliance (18). Sakr et al., (19) reported in 2020 that in order to control the growing problem of antibiotic resistance in Lebanon, there is an urgent need for awareness campaigns on appropriate use of antibiotics in parallel with rigorous surveillance system for antimicrobial use and resistance.

In a cross-sectional study conducted by Moghnieh et al., (20) on antimicrobial susceptibility pattern of bacterial isolates from hospitalized patients in 13 Lebanese hospitals over two consecutive years (2015 - 2016), an overall decrease in susceptibility of bacterial isolates to different antibiotics among clinical GNB and GPB involved in various infections was reported. To the best our knowledge, there are no reports of antibiotic resistance rates of isolated strains in Lebanese hospitals during the last two years (2018 and 2019). The objective of this current study therefore is to investigate the changing pattern of antibiotic resistance of bacteria pathogens isolated from hospitalized patients with clinical infections in two Lebanese hospitals over this period.

Materials and method:

Study setting and population

This retrospective study was conducted in two hospitals (in Haroun and Bekaa) in Lebanon with about 70% of bacterial isolates from hospitalized patients in Internal Medicine department and 30% from patients in Surgical and Outpatient deparments, Intensive Care Unit (ICU) and intubated patients in Coronary Care Unit.

Specimen types

Bacterial isolates were recovered from routine clinical specimens such as urine, sputum, tracheal aspirates, pus, abscess, blood, wounds and rectal specimens, which were

collected from patients of different age groups and gender.

Culture isolation and identification of bacterial isolates from specimens

All clinical samples were routinely cultured in the laboratory of the two hospitals on standard agar media appropriate for each specimen, and these included Blood agar, Salmonella Shigella (SS) agar, MacConkey agar, Columbia agar, Chocolate agar, Schaedler agar, and Thiosulfate Citrate Bile salt Sucrose (TCBS) agar, using standard microbiological methods.

Antibiotic susceptibility testing

The antibiotic susceptibility testings (AST) of the isolates were routinely performed against anti-bacterial agents (as shown in Table 1) by the disc diffusion method, and zone diameters of inhibition interpreted according to the EUCAST/2019, CLSI/2018 or WHO/MOH/ SOP susceptibility testing/2018 guidelines. The ASTs were performed using Muller-Hinton (MH) agar except for streptococcal (enterococcal) isolates which were performed on MH media supplemented with blood.

The discs contents used for the AST were; trimetropim/sulfamethoxazole 1.25/23.75 µg, ciprofloxacin 5µg, ofloxacin 5µg, pefloxacin 5µg, penicillin G 10µg, imipenem 10µg, gentamicin 10µg, colistin 10µg, tigecycline 15µg, amoxicillin /clavulinic acid 20/10µg, piperacillin/tazo-bactam 100/10µg, cefepime 30µg, cefoxitin 30 µg, cefotaxime 30µg, cefuroxime 30µg, cefotaxime 30µg, ceftazidime 30µg, amikacin 30 µg, clindamycin 30µg, erythromycin 30µg, vancomycin 30µg, teicoplanin 30µg, fosfomycin 300µg, and nitrofurantoin 300µg.

Statistical analysis of data

The bacterial identification and susceptibility data were tabulated in Excel spreadsheets. The resistance rates to individual antibiotic were calculated for every bacterial isolate by year of isolation. The mean percentage resistance of each isolate to all tested antibiotics were also calculated. Yearly comparisons were performed using Z-test after checking the applicability conditions. When comparing results from the two different years, $p<0.05$ was considered statistically significant if at least one value was different from the other.

Table 1: Antibacterial agents with their respective classes used in this study

Antibacterial drug class	Antibacterial drug	Disc content (µg)
Aminoglycoside	Gentamicin	10
	Amikacin	30
Beta-lactam	Amoxicillin/clavulanic acid	20/10
	Cefepime	30
	Cefotaxime	30
	Cefoxitin	30
	Ceftazidime	30
	Ceftriaxone	30
	Cefuroxime	30
	Imipinem	10
	Piperacillin/tazobactam	100/10
	Penicillin G	10
Glycopeptide	Vancomycin	30
	Teicoplanin	30
Fluoroquinolone	Ciprofloxacin	5
	Ofloxacin	5
	Pefloxacin	5
Fosfomycin	Fosfomycin	300
Inhibitor of folate pathway	Sulfamethoxazole/trimethoprim	1.25/23.75
Lincosomide	Clindamycin	30
Macrolide	Erythromycin	30
Nitrofuran	Nitrofurantion	300
Oxazolidinone	Linezolid	30
Polymyxin E	Colistin	10
Glycycyline	Tigecycline	15

Ethical consideration

The study was reviewed and approved by the Lebanese International University Institutional Review Board (IRB) ethical committee (Reference LIUIRB-200305-SS2). All collected data were purely based on micro-organisms, and there was no need for a written informed consent as all the patients were anonymous and no personal information was used in the study.

Results:

A total of 1698 bacterial isolates from patients with clinical infections in the two hospitals were recovered in the year 2018 and 2019. As shown in Table 2, there were 1486 (87.7%) Gram-negative bacterial isolates and 212 (12.3%) Gram-positive isolates (12.3%). The Gram-negative isolates are distributed as follows; 982 *E. coli* (66.1%), 114 *K. pneumoniae* (7.7%), 99 *P. mirabilis* (6.7%), 198 *P. aeruginosa* (13.3%) and 93 *Enterobacter* sp. (6.3%). Among the Gram-positive isolates; 67 (31.6%) were *E. faecalis* and 145 (67.4%) were coagulase positive staphylococci (mainly *S. aureus*).

As presented in Table 3a, a total of 982 *E. coli* isolates were recovered; 465 isolates in 2018 and 517 isolates in 2019. Their resistance pattern to 18 antibiotics showed that between 44% and 68% isolates were resistant to amoxicillin/clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, colistin, pefloxacin and sulfamethoxazole/trimethoprim. However, compared to 2018, the resistance rate of the isolates in 2019 to cefoxitin, ceftazidime, colistin, fosfomycin,

Table 2: Frequency of bacterial pathogens isolated from patients in two Lebanese hospitals (2018-2019)

Bacterial isolates	Number (%)
Gram-negative isolates	**1486 (87.5)**
Escherichia coli	982 (66.1)
Klebsiella pneumoniae	114 (7.7)
Proteus mirabilis	99 (6.7)
Pseudomonas aeruginosa	198 (13.3)
Enterobacter sp.	93 (6.3)
Gram positive isolates	**212 (12.5)**
Enterococcus faecalis	67 (31.6)
Coagulase positive staphylococci	145 (68.4)

Table 3a: Resistance rates of Gram-negative isolates in 2018 and 2019: *Escherichia coli*

Antibacterial agent	*Escherichia coli*			*p*-value
	Percentage of resistance to antibacterial drugs			
	2018 (n=465)	2019 (n=517)	Both years (n=1037)	
Amikacin	3.2	1.6	2.2	0.0745
Amoxicillin/clavulanic acid	58.7	63.3	57.9	0.1502
Cefepime	36.1	41.6	36.9	0.0768
Cefotaxime	45.4	49.5	45.0	0.1929
Cefoxitin	45.4	29.8	35.2	**0.0000[a]**
Ceftazidime	78.3	66.7	68.4	**0.0001[a]**
Ceftriaxone	46.2	50.9	46.1	0.1458
Cefuroxime	56.5	58.2	54.4	0.6017
Ciprofloxacin	50.8	45.6	45.5	0.1087
Colistin	61.1	35.0	44.8	**0.0000[a]**
Fosfomycin	4.9	2.1	3.3	**0.0132[a]**
Gentamicin	23.6	21.9	21.5	0.4932
Imipinem	1.3	0.8	1.0	0.4081
Nitrofurantion	7.5	9.5	8.1	0.2632
Pefloxacin	76.8	62.1	65.4	**0.0000[a]**
Piperacillin/tazobactam	17.0	9.9	12.5	**0.0007[a]**
Sulfamethoxazole/trimethoprim	53.8	53.4	50.7	0.9056
Tigecycline	1.9	1.2	1.4	0.3098

n=number, a = *p* value < 0.05 respresent significant difference in % between 2018 and 2019

Table 3b: Resistance rate of Gram-negative isolates in 2018 and 2019: *Klebsiella pneumoniae*

Antibiotic	***Klebsiella pneumoniae***			***p*-value**
	Percentage of resistance to antibacterial drugs			
	2018 (n=74)	2019 (n=40)	Both years (n=114)	
Amikacin	1.4	0.0	0.9	0.4602
Amoxicillin/clavulanic acid	50.0	50.0	50.0	1
Cefepime	24.3	27.5	25.4	0. 7102
Cefotaxime	37.8	32.5	36.0	0.5708
Cefoxitin	44.6	20.0	36.0	**0.0090[a]**
Ceftazidim	83.8	62.5	76.3	**0.0107[a]**
Ceftriaxone	39.20	35.0	37.7	0.6596
Cefuroxime	47.3	40.0	44.7	0.4545
Ciprofloxacin	23.0	27.5	24.6	0.5920
Colistin	71.6	42.5	61.4	**0.0023[a]**
Fosfomycin	14.9	2.5	10.5	**0.0400[a]**
Gentamicin	16.2	12.5	15.0	0.5949
Imipinem	4.1	0.0	2.6	0.1968
Nitrofurantion	33.8	42.5	36.8	0.3571
Pefloxacin	82.4	55.0	72.8	**0.0016[a]**
Piperacillin/tazobactam	17.6	7.5	14.0	0.1397
Sulfamethoxazole/trimethoprim	51.3	35.0	45.6	0.0943
Tigecycline	1.3	0.0	0.9	0.4602

n=number, a = *p* value < 0.05 respresent significant difference in % between 2018 and 2019

pefloxacin and piperacillin/tazobactam decreased by 34.4%, 14.8%, 42.7%, 56.9%, 19.1% and 41.9% respectively. Also, *E. coli* isolates over the 2 years exhibited considerable susceptibility to amikacin, imipenem, and tigecycline. Indeed, less than 3% of *E. coli* isolates were resistant to each one of these antibiotics, while 8.6% of the isolates in 2018/19 were resistant to nitrofurantoin.

The results of resistance of 114 *K. pneumoniae* isolates to 18 different antibiotics assessed for 2018 and 2019 are presented in Table 3b, which shows a significant decrease in 2019 of *K. pneumoniae* resistance to 5 antibiotics; cefoxitin, ceftazidime, colistin, fosfomycin and pefloxacin by 55.1%, 25.4%, 49.3%, 83.2% and 33.3% respectively. Fifty percent of the 114 isolates were resistant to amoxicillin/clavulanic acid, 76.3% to ceftazidime, 44.7% to cefuroxime, 61.4% to colistin and 72.8% to pefloxacin. The resistance of the 114 isolates to amikacin, imipenem and tigecycline did not exceed 3% while between 10 to 14% were resistant to fosfomycin, gentamicin and piperacillin/tazobactam.

The data on the 99 *P. mirabilis* isolates in the study as shown Table 3c, showed that 2% were resistant to imipenem, 5% to amikacin and piperacillin/tazobactam, and 46% to amoxicillin/clavulanic acid. Between 60 and 93% of these isolates were resistant to ceftazidime, colistin, nitrofurantoin, pefloxacin, sulfamethoxazole/trimethoprim and tigecycline. In 2019, there was a significant decrease in resistance of *P. mirabilis* isolates by 54.6%, 18%, 65.1%, 46.7%, 34% and 35.9% respectively to ceftazidime, colistin, fosfomycin, nitrofurantoin, pefloxacin and tigecycline, compared to 2018.

Regarding the resistance of the 93 *Enterobacter* isolates, as shown in Table 3d, 3% of the isolates were resistant to amikacin, ertapenem, imipenem and tigecycline. Between 11 and 16% were resistant to ciprofloxacin, cefepime, gentamicin and piperacillin/tazobactam. Most of the isolates were resistant to amoxicillin/clavulanic acid, cefoxitin, ceftazidime, colistin and pefloxacin, with resistance rate varying between 84 and 98% of the total isolates. About 250% more isolates were resistant to piperacillin/tazobactam in 2019 compared to 2018, while in 2019, the resistance of the isolates to colistin and pefloxacin decreased by 26.6% and 23.4% respectively, compared to the year 2018.

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Table 3c: Resistance rate of Gram-negative isolates in 2018 and 2019: *Proteus mirabilis*

Antibiotic	***Proteus mirabilis***			***p*-value**
	Percentage of resistance to antibacterial drugs			
	2018 (n=60)%	2019 (n=39)%	All years (n=99)%	
Amikacin	7	3	5	0.3623
Amoxicillin/clavulanic acid	52	38	46	0.1980
Cefepime	10	10	10	0.9670
Cefotaxime	12	13	12	0.8635
Cefoxitin	28	23	26	0.5614
Ceftazidim	97	44	76	**0.0000[a]**
Ceftriaxone	13	13	13	0.9411
Cefuroxime	27	28	27	0.8666
Ciprofloxacin	37	31	34	0.5459
Colistin	100	82	93	**0.0006[a]**
Fosfomycin	43	15	32	**0.0036 [a]**
Gentamicin	30	46	36	0.1025
Imipinem	3	0	2	0.2493
Nitrofurantion	92	49	75	**0.0000[a]**
Pefloxacin	97	64	84	**0.0000[a]**
Piperacillin/tazobactam	7	3	5	0.3623
Sulfamethoxazole/trimethoprim	60	59	60	0.9190
Tigecycline	92	59	79	**0.0001[a]**

n=number, a = *p* value < 0.05 respresent significant difference in % between 2018 and 2019

Table 3d: Resistance rate of Gram-negative isolates in 2018 and 2019: *Enterobacter* spp

Antibiotic	***Enterobacter* spp**			***p*-value**
	Percentage of resistance to antibacterial drugs			
	2018 (n=54)	2019 (n=39)	Both years (n=93)	
Amikacin	4	3	3	0.7588
Amoxicillin/clavulanic acid	93	92	92	0.9590
Cefepime	15	18	16	0.6851
Cefotaxime	22	26	24	0.7018
Cefoxitin	98	97	98	0.8152
Ceftazidime	94	82	89	0.0569
Ceftriaxone	22	28	25	0.5093
Cefuroxime	63	49	57	0.1709
Ciprofloxacin	15	5	11	0.1367
Colistin	94	69	84	**0.0011[a]**
Ertapenem	2	5	3	0.3775
Gentamicin	19	8	14	0.1373
Imipinem	2	5	3	0.3775
Pefloxacin	94	72	85	**0.0025[a]**
Piperacillin/tazobactam	6	21	12	**0.0275[a]**
Sulfamethoxazole/trimethoprim	24	28	26	0.6532
Tigecycline	6	0	3	0.1345

n=number, a = *p* value < 0.05 respresent significant difference in % between 2018 and 2019

Among the 198 *P. aeroginosa* isolates (Table 3e), only 2% were resistant to colistin and 11% to amikacin. Between 19% and 24% were resistant to cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin and piperacillin/tazobactam. In 2019, the decrease of the resistance to amikacin and imipenem was respectively 66.7% and 65.5%.

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Table 3e: Resistance rates of Gram-negative isolates in 2018 and 2019: *Pseudomonas aeruginosa*

Antibiotic	***Pseudomonas aeroginosa***			***p*-value**
	Percentage of resistance to antibacterial drugs			
	2018 (n=105)	2019 (n=93)	Both years (n=198)	
Amikacin	15	5	11	**0.0245[a]**
Cefepime	21	23	22	0.7815
Ceftazidime	22	16	19	0.3030
Ciprofloxacin	25	20	23	0.4678
Colistin	1	2	2	0.4909
Gentamicin	19	23	21	0.5403
Imipinem	29	10	20	**0.0008[a]**
Levofloxacin	26	22	24	0.4872
Piperacillin/tazobactam	20	20	20	0.9400

n=number, a = *p* value < 0.05 respresent significant difference in % between 2018 and 2019

Table 4a: Resistance rate of Gram-positive bacteria isolates in 2018 and 2019: *Enterococcus faecalis*

Antibiotic	***Enterococcus faecalis***			*p*-value
	Percentage of resistance to antibacterial drugs			
	2018 (n=29)	2019 (n=38)	Both years (n=67)	
Ciprofloxacin	6. 9	94.7	56. 7	**0.0000[a]**
Erythromycin	65. 5	89.5	79. 1	**0.0168[a]**
Gentamicin	3. 5	92.1	53. 7	**0.0000[a]**
Linezolid	0	0	0	1
Teicoplanin	13.8	34.2	25.4	0.0570
Tigecycline	0	31.6	17. 9	**0.0008[a]**
Vancomycin	24. 1	36. 8	31. 3	0.2667

n=number, a = *p* value < 0.05 respresent significant difference in % between 2018 and 2019

Table 4b: Resistance rate of Gram-positive bacteria isolates in 2018 and 2019: Coagulase Positive Staphylococci

Antibiotic	**Coagulase positive staphylococci**			***p*-value**
	Percentage of resistance to antibacterial drugs			
	2018 (n=82)	2019 (n=63)	Both years (n=145)	
Amoxicillin/clavulanic acid	30.5	25. 4	28	0.4998
Cefoxitin	87. 8	46. 0	70	**0.0000[a]**
Ceftriaxone	54.9	34. 9	46	**0.0168[a]**
Cefuroxime	87. 8	50.8	72	**0.0000[a]**
Ciprofloxacin	9. 8	19.1	14	0.1077
Clindamycin	24. 4	15.9	21	0.2094
Erythromycin	18.3	22. 2	20	0.5576
Linezolid	0	0	0	1
Ofloxacin	12. 2	19.1	15	0.2542
Penicillin G	26. 8	58. 7	41	**0.0001[a]**
Sulfamethoxazole/trimethoprim	9.8	6.4	8	0.4604
Teicoplanin	0	1.6	1	0.2522
Vancomycin	23.2	7.9	17	**0.0144[a]**

n: number, a: *p* value <0.05 represents significant difference in % between 2018 and 2019.

Analysis of the resistance of 67 *E. faecalis* isolates to 7 different antibiotics (as shown in Table 4a) clearly revealed a major increase of the resistance of these isolates between 2018 and 2019 to four antibiotics. Compared to the 29 isolates in 2018, the 38 isolates in 2019 showed an increase of their resistance by 1273% to ciprofloxacin, by 36.6% to erythromycin, and by 2569.8% to gentamicin (Table 5). This was added to the fact that in 2018, none of the isolates was resistant to the tigecycline, compared to 31% of isolates in 2019 showing resistance to the same antibiotics. None of the isolates in 2018 and 2019 was resistant to linezolid.

The antibiotic resistance patterns of the 145 coagulase positive staphylococci (mainly *S. aureus*) are summarized in Table 4b, with 82 isolates in 2018 and 63 isolates in 2019. Over these two years, no resistance to linezolid was detected, and only 1% of patients developed resistance to teicoplanin. Between 8% and 21% were resistant to ciprofloxacin, clindamycin, erythromycin, ofloxacin, and sulfamethoxazole/ trimethoprim. Approximately 70%, 46% and 41% were respectively resistant to cefoxitin, ceftriaxone and penicillin G. The resistant rates in 2019 to amoxicillin/clavulanic acid, cefoxitin, ceftriaxone, cefuroxime and vancomycin decreased by 16.7%, 47.6%, 36.4%, 42.2% and 65.7% respectively, compared to 2018. The resistant rate to penicillin G however increased by 123% in 2019, compared to 2018 (Table 5).

Table 5: Antibiotic resistance rates and trends among bacterial isolates in two Lebanese hospital (2018 and 2019)

Isolate	Bacterial species	Lowest and highest resistance rate of total isolates		Trends in resistance to antibiotics between 2018- 2019			
		≤5% of resistance	≥60% of resistance	Significant decrease of the resistance to	Decrease by:	Significant increase of the resistance to	Increase by:
GNB isolates	*E. coli*	Amikacin Fosfomycin Imipinem Tigecycline	Amoxicillin/clavulanic acid Ceftazidime Pefloxacin	Cefoxitin Ceftazidime Colistin Fosfomycin Pefloxacin Piperacillin/tazobactam	14.7 - 56.9%	None	NA
	K. pneumoniae	Amikacin Imipinem Tigecycline	Ceftazidime Colistin Pefloxacin	Cefoxitin Ceftazidime Colistin Fosfomycin Pefloxacin	25.4 - 83.2%	None	NA
	P. mirabilis	Amikacin Imipinem Piperacillin/tazobactam	Ceftazidime Colistin Nitrofurantion Pefloxacin Sulfamethoxazole/trimethoprim Tigecycline	Ceftazidime Colistin Fosfomycin Nitrofurantion Pefloxacin Tigecycline	18 - 65.1%	None	NA
	Enterobacter spp	Amikacin Imipinem Ertapenem Tigecycline	Amoxicillin/clavulanic acid Cefoxitin Ceftazidime Colistin Pefloxacin	Colistin Pefloxacin	23.3 - 26.6%	Piperacillin/tazobactam	250%
	P. aeruginosa	Colistin	None	Amikacin Imipinem	65.5 - 66.7%	None	NA
GPB isolates	*E. faecalis*	Linezolid	Erythromycin	None	NA	Ciprofloxacin Erythromycin Gentamycin Tigecycline	36.5-2569%
	CoPS	Linezolid Teicoplanin	Cefuroxime	Amoxicillin/clavulanate Cefoxitin Ceftriaxone Cefuroxime Vancomycin	16.7 - 65.7%	Penicillin G	123%

NA= Not applicable; GNB = Gram negative bacteria; GPB = Gram positive bacteria; CoPS = Coagulase Positive Staphylococci; *E. coli* = *Escherrichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*; *P. mirabilis* = Proteus mirabilis; *P. aeruginosa* = *Pseudomonas aeruginosa*; *E. faecalis* = *Enterococcus faecalis*

Discussion:

In this study, we aimed to compare our findings on antibiotic resistance pattern of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Enterobacter* sp, *P. aeruginosa*, *E. faecalis,* and *S. aureus* isolates from hospitalised patients in Lebanon in 2018 and 2019 with national and international data. The irrational use of antibiotics, easy access, low cost of many antibiotics and absence of an efficient national antimicrobial resistance surveillance system have all contributed to the increase of the resistance of many pathogenic bacterial species to different antibiotics. Our findings however showed a significant decrease (14.8 - 56.9%) of *E. coli* resistance in 2019 compared to 2018 against cefoxitin, ceftazidime, colistin, fosfomycin, pefloxacin, and piperacillin/tazobactam, while resistance rate was lowest (less than 3.5%) against imipinem, amikacin, and tigecycline. This pattern is similar to what was reported between 2011 and 2013 (18), but more than 60% of our isolates were resistant to amoxicillin/clavulanic acid and ceftazidime, which is significantly higher compared to 2011 and 2013 (which reported 39 and 29% respectively). In 2015/2016, resistance rate of *E. coli* compared to 2011/2013 in Lebanon increased to most of the tested antibiotics (20). This increase could have been caused by selective pressure of irrational use, promoted by easy access to these antibiotics, which are available over-the-counter in many Lebanese community. This highlights the urgent need for awareness and national educational campaigns to increase the knowledge and ameliorate the attitude and practice towards antibiotic use.

In Gabon, *E. coli* isolates recovered between 2009 and 2012 were susceptible to ceftriaxone, ciprofloxacin, and gentamicin (21). However, a similar study conducted in Ethiopia between 1990 and 2013 showed that most Gram-negative isolates were relatively resistant to most of the tested antibiotics (22). Between 2012 and 2016 in Nepal (23), 75% of *E. coli* isolates from children with urinary tract infection were resistant to cefotaxime, which is higher compared to the 37.2% reported in our study, but the resistance rates of *E. coli* to gentamicin and amikacin were similar. Compared to a study conducted in Zambia between 2016 and 2018 (24), our *E. coli* isolates demonstrated higher resistance to ceftazidime but lower resistance to ciprofloxacin. In Iran, *E. coli* isolates between 2015 and 2016 (25), demonstrated higher resistance to ceftriaxone, ciprofloxacin gentamicin, and imipinem but lower resistance to ceftazidime and amikacin compared to the findings in our study. Smilarly in Yucatan city, in Mexico, between 2016 and 2018 (26), higher resistance rates were seen in *E. coli* isolates compared to the rates reported in our current study.

The *K. pneumoniae* isolates in our study showed low resistance (less than 5%) to amikacin, fosfomycin, imipinem, and tigecycline and high resistance (more than 60%) to amoxicillin/clavulanic acid, ceftazidime and pefloxacin. When compared to the year 2018, the resistance rate decreased in 2019 by 25.4 - 83.2% to cefoxitin, ceftazidime, colistin, fosfomycin and pefloxacin. *K. pneumoniae* isolates in our study showed significantly less resistance to ciprofloxacin, ceftriaxone, and gentamicin compared to *K. pneumoniae* isolates reported between 2009 and 2012 in Gabon (21). In Ethiopia, the resistance rate of *K. pneumoniae* isolates between 1990 and 2013 was considerably lower against cefoxitin compared to the isolates in our current study, but higher resistance to ceftriaxone, amoxicillin/clavulanic acid, ciprofloxacin, gentamicin and trimethoprim/sulfamethoxazole (22). In Iran between the year 2015 and 2016, *K. pneumoniae* isolates compared to our study showed a lower resistance rate to all the antibiotics tested by Hasani et al., (25) except for ceftazidime. Compared to the findings of Uc-Cachon et al., (26) in Mexico between 2016 and 2018, our isolates showed a significantly lower resistant rates to cefepime, cefuroxime, gentamicin, imipenem, piperacillin/tazobactam and trimethoprim/sulfamethoxazole, but similar resistance rate to ciprofloxacin. According to the WHO and based on the tests performed on 30 isolates per nation, Prestinaci et al., (11) reported that 17.4% of *K. pneumoniae* isolates Eastern Mediterranean countries were resistant to carbapenems and third generation cephalosporins, while 36% of isolates from South East Asia and regions of America were resistant, and almost 60% for the European region.

Our study revealed that amikacin, imipinem, and piperacillin/tazobactam are the antibiotics to which *P. mirabilis* isolates were less resistant to compared to ceftazidime, colistin, nitrofurantion, pefloxacin, sulfamethoxazole/trimethoprim, and tigecycline to which these isolates were most resistant to. A

significant decrease (18 - 65.1%) in *P. mirabilis* resistance to ceftazidime, colistin, nitrofurantoin, pefloxacin, and tigecycline was reported in our study. This significant decrease could be due to the fact that most of these antibiotics are not accessible in pharmacies but are administrated only in hospitals. Our data showed significantly lower resistance rate to amoxicillin/clavulanic acid, ceftriaxone, ciprofloxacin, gentamicin, and sulfamethoxazole/trimethoprim compared to *P. mirabilis* isolates from the study in Ethiopia (22).

Regarding the resistance patterns of *Enterobacter* isolates in our study, less than 5% were resistant to amikacin, imipinem, ertapenem, and tigecycline, but more than 60% were resistant to amoxicillin/clavulanic acid, cefoxitin, ceftazidime, colistin and pefloxacin. The isolates in 2019 showed a significant decrease (23.3% - 26.6%) in resistance rates to colistin and pefloxacin, but significant increase (250%) to piperacillin/tazobactam. Clearly, *Enterobacter* isolates in the current study showed a lower resistance rate to ciprofloxacin, gentamicin and trimethoprim/sulfamethoxazole but higher resistance rate to ceftriaxone and amoxicillin/clavulanic acid, compared to the isolates in Ethiopian study (22), which could also be explained by the easy access and relative low cost of these two antibiotics in Lebanaon. In Romania, Golli et al., (27) in 2017 reported that *Enterobacter* isolates showed considerably high resistance rate to cefepime, ceftriaxone, ciprofloxacin, imipenem, but low resistance to ceftazidime.

Our data confirmed that less than 5% of *P. aeruginosa* isolates were resistant to colistin, and resistance to amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipinem, levofloxacin, and piperacillin/tazobactam did not exceed 24% (varied between 11% to amikacin and 24% to levofloxacin). There was a decrease in 2019 in the resistance rate to amikacin and imipenem by 65.5% and 66.7% compared to 2018. The low incidence of infections caused by *P. aeroginosa* in the Lebanese community could support the significantly lower resistance of this pathogenic bacteria to the tested antibiotics. No significant difference in the pattern of antibiotic resistance of *P. aeruginosa* in our study was observed while comparing the results of the two studies done in Lebanon by Chamoun et al., (18) between 2011 and 2013, and by Moghnieh and colleagues (20) between 2015 and 2016. A five-year study conducted in Saudi Arabia between 2013 and 2017 (28) reported high resistance rate in *P. aeruginosa* isolates to β-lactams, fluoroquinolones and aminoglycosides. The study conducted in India between the 2013 and 2015 reported a similar resistance rate of *P. aeruginosa* to imipenem, comparable to our study. In the study conducted in Iran in 2015 and 2016 (25), *P. aeruginosa* isolates were more resistant to amikacin, ceftazidime, ciprofloxacin, gentamicin, and levofloxacin, compared to the isolates in our study. As reported by Reta et al., (22) in their retrospective study in Ethiopia between the year 1990 and 2013, *P. aeruginosa* isolates were significantly more resistant to ciprofloxacin than our isolates, but similar resistance rates were reported to gentamicin. The German study (29) conducted between 2013 and 2018 reported similar resistance rates of *P. aeruginosa* to imipenem, ciprofloxacin and piperacillin/tazobactaman as the isolates in our study, but the German *Pseudomonas* isolates were less resistant to ceftazidime. In Romania (27), *P. aeruginosa* isolates in 2017 were more resistant to piperacillin/tazobactaman, ciprofloxacin and ceftazidime compared to our isolates. In Mexico, the resistance rates of *P. aeruginosa* isolates reported between 2016 and 2018 (26) were higher to amikacin, ceftazidime, cefepime, ciprofloxacin, imipenem, gentamicin, levofloxacin, and piperacillin/tazobactam, compared to our isolates.

While analyzing the *E. faecalis* isolates, our results showed a significant increase (36.6% - 2569.8%) in the resistance rates of isolates in 2019 to ciprofloxacin, erythromycin, gentamicin, and tigecycline, compared to 2018**,** which could be explained by the overuse due to wide availability and low cost of macrolides and β-lactam antibiotics in Lebanon. In Ethiopia, the study by Reta et al., (22) showed lower rates of resistance of *E. faecalis* isolates compared to our isolates while similar resistance rate to erythromycin was reported. In Germany, as shown by Rothe et al., (29), *E. faecalis* isolates recovered between the year 2013 and 2018 showed no resistance (0%) to vancomycin and linezolid while 31.3% of our tested isolates were resistant to vancomycin though none to linezolid.

Our study also showed that coagulase positive staphylococci CoPS (mostly *S. aureus*) showed no resistance (0%) to linezolid and only 1% was resistant to teicoplanin. The highest resistance rates were to cefoxitin (70%) and cefuroxime (72%). This discrepancy in resistance rates could be correlated with overuse of the widely available β-lactam drugs for treatment of *S. aureus* infections whereas linezolid

and teicoplanin use is very limited and restricted to hospitalised patients. Cefoxitin is a surrogate phenotypic marker of methicillin resistance in *S. aureus*, which implies that the phenotypic MRSA rate in our study is 70%, a relatively high rate for this pathogen in Lebanon. Approximately 14%, 15% and 17% of the CoPS isolates were resistant to ciprofloxacin, ofloxacin and vancomycin respectively, 20% to erythromycin, 21% to clindamycin, 41% to penicillin G, and 46% to ceftriaxone. Between the year 2011 to 2019, the resistance of *S. aureus* isolates to clindamycin, erythromycin and trimethoprim/sulfamethoxazole was relatively stable whereas the rate increased for vancomycin (18,19,20). However, it should be noted that by the CLSI guideline, vancomycin disc diffusion is no longer used to assess phenotypic resistance of clinical *S. aureus* isolates to vancomycin, haven been replaced with vancomycin MIC determination by broth dilution or E-test methods. In Ethiopia, between 1990 and 2013, Reta et al., (22) reported that resistance rate of *S. aureus* isolates was lower for cefoxitin, but higher for amoxicillin/clavulanic acid, erythromycin, trimethoprim/sulfamethoxazole, ceftriaxone and ciprofloxacin. In Iran, between 2015 and 2016 (25), *S. aureus* isolates showed lower resistance to ceftriaxone compared to the isolates in our study but similar pattern of resistance to ciprofloxacin and amikacin. In the German study by Rothe et al., (29) between 2013 and 2018, 3.3% of *S. aureus* isolates were resistant to cefuroxime and ceftriaxone compared to 72% and 46% in our isolates, and fewer isolates were also resistant to ciprofloxacin compared to our isolates. All *S. aureus* isolates in the German study were susceptible to linezolid as in our study. However, the resistance rate to vancomycin in the German study was 0% while the rate was 17% in our study. In comparing the resistance pattern of *S. aureus* isolates reported by the Greek study conducted between 2010 and 2015 (30) to our current data, it was clear that our isolates were more resistant to cefoxitin, trimethoprim/sulfamethoxazole and vancomycin, while they were less resistant to erythromycin and penicillin G, but similar resistance pattern to teicoplanin and clindamycin.

It has been reported that more than 2.8 million infections and 35,000 deaths caused by antibiotic resistant bacteria and fungi occurs yearly in the United States (31). If no action is taken, drug-resistant infections could cause 10 million deaths each year by 2050 (32). Such infections have serious burden on the economy. Indeed, the cost of AMR across the globe is extremely high though differs between countries (33). Resistance rates are generally higher in low-income countries compared to middle and upper income countries (34).

In Lebanon, the use of antibacterial guidelines should be among the priorities in line with the practice in advanced countries (35). Effective research directed at not only human health sector, but also across animal health and environment sectors, should be prioritised since they are inter-connected (36). This is similar to the action plan adopted by the United States since the year 2015 (37). Global partnerships between industry, researchers and academia are needed to develop new antibiotics such as the European program, New Drugs 4 Bad Bugs (ND4BB) (38), and the Combating Antibiotic-Resistant Bacteria (CARB-X) program, which since its establishment in 2016, is investing in 75 projects around the world and accelerating the global antibacterial innovations (39). A reason to hope of a better future is the fact that approximately 41 new antibiotics (as of March 2017) are in different phases of clinical development aimed at antimicrobial therapy of serious bacterial infections (40).

The present study has some limitations. First, only two hospitals were included in the study, which may not represent the total picture of bacterial AMR in Lebanon. Another limitation is the lack of descriptive details related to the source of the isolates, but we are aiming to assess the multi-drug resistance details among the bacterial isolates in the future. Also, being a retrospective study, we have to rely on the conventional phenotypic methods used for routine bacteria identification and susceptibility tests by the laboratories, which are are not as accurate as genotypic methods. However, our study could motivate the implementation of a national surveillance for antimicrobial resistance in Lebanon.

Conclusion:

Overuse of antibiotics, which is possible because of easy access to these drugs, is among the major factors underlying emergence and increasing antibiotic resistance in Lebanon. We agree with the 2020 recommendation of Sakr et al., (19) on the need to promote awareness among students, especially in the non-health related majors, and to enact governmental policy that will limit access to antibiotics.

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Conflict of interest:

Authors declared no conflict of interest

Contributions of authors:

AM and TK contributed equally to the study

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