ARTICLE ORIGINAL / ORIGINAL ARTICLE

A REFLECTION ON BACTERIAL RESISTANCE TO ANTIMICROBIAL AGENTS AT A MAJOR TERTIARY CARE CENTER IN LEBANON OVER A DECADE

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ABSTRACT • BACKGROUND : Antimicrobial resistance has been inflecting deleterious health and economic consequences locally and globally. This study addresses the patterns and trends of bacterial resistance to antimicrobial agents over a decade, at a major tertiary care center in Beirut.

METHODS : Data on bacterial susceptibility patterns at the CAP accredited Clinical Microbiology Laboratory is analyzed from January 2000 to November 2011, along with related different studies conducted during this period.

RESULTS : Increasing rates of ESBL-producing isolates were noted for *Escherichia coli*, *Klebsiella pneumoniae, Salmonella* spp. and *Shigella* spp.

Resistance to carbapenems remains problematic in *Acinetobacter* spp. and *Pseudomonas aeruginosa*, and started emerging in *E. coli* and *K. pneumoniae*.

Tigecycline and colistin maintained excellent activity against most ESBL and carbapenem resistant bacteria relevant to the treatment by these agents.

Resistance to quinolones is being encountered in *Streptococcus pneumoniae, Haemophilus influenzae, Salmonella* spp. and *Shigella* spp.

Methicillin resistant *Staphylococcus aureus* (MRSA), though remaining relatively high, showed decreasing trends of resistance, while vancomycin maintain uniform activity. Rare and sporadic vancomycin resistant strains in enterococci are encountered.

Macrolide and clindamycin increasing rates of resistance is noted in *S. pneumoniae*, group A streptococci, *S. aureus*, viridans streptococci and some others.

CONCLUSION : Physicians should be aware of the local epidemiology of antimicrobial resistance to properly guide the initial therapy. These resistance problems can be attributed to uncontrolled use of antimicrobial agents, thus, highlighting the need for antimicrobial stewardship to curb this threat.

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Araj GF, Avedissian AZ, Ayyash NS, Bey HA, El Asmar RG, Hammoud RZ, Itani LY, Malak MR, Sabai SA. Une réflexion sur la résistance bactérienne aux antibiotiques dans un centre de soins tertiaire au Liban au cours d'une décennie. J Med Liban 2012 ; 60 (3) :125-135.

RÉSUMÉ • HISTORIQUE : L'accroissement progressif de la résistance bactérienne aux antibiotiques se répercute, localement et globalement, aux niveaux sanitaire et économique. Cette étude vise à étudier les caractéristiques et l'évolution de la résistance bactérienne aux agents antimicrobiens au cours d'une décennie dans un centre de soins tertiaire à Beyrouth.

MÉTHODES : De janvier 2000 à novembre 2011 des données concernant la sensibilité bactérienne aux antibiotiques dans un laboratoire de microbiologie clinique, accrédité par le CAP (College of American Pathologists), ont été analysées ainsi que les études publiées dans ce domaine au cours de cette période.

RÉSULTATS : On a noté un taux croissant de souches produisant une ß-lactamase à spectre étendu (BLSE) pour *Escherichia coli, Klebsiella pneumoniae, Salmonella* spp. et *Shigella* spp.

La résistance aux carbapénèmes reste problématique en cas d'*Acinetobacter* spp. et de *Pseudomonas aeruginosa*, et commence à émerger en cas d'*E. coli* et *K. pneumoniae*.

Tigecycline et colistine maintiennent une excellente activité contre la plupart des bactéries produisant une BLSE ou résistant aux carbapénèmes et pouvant être traitées par ces agents.

Une résistance aux quinolones est observée en cas de *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Salmonella* spp. et *Shigella* spp.

Le *Staphylococcus aureus* résistant à la méthicilline (SARM) garde une incidence relativement élevée, mais sa résistance tend à décroître, alors que la vancomycine maintient la même activité. Quelques rares souches d'entérocoques sont résistantes à la vancomycine.

Des taux accrus de résistance aux macrolides et à la clindamycine sont notés en cas de *S. pneumoniae*, streptocoques du groupe A, *S. aureus*, streptocoques viridans et quelques autres souches.

CONCLUSION : Les médecins doivent connaître l'épidémiologie locale de la résistance bactérienne avant de prescrire toute antibiothérapie. La résistance bactérienne aux antibiotiques est en grande partie attribuée à l'usage anarchique des antimicrobiens. Ceci est alarmant et nécessite un contrôle des prescriptions d'agents antimicrobiens pour endiguer cette menace.

INTRODUCTION

The clinical microbiology division is the primary gate keeper of infectious etiologies at hospitals and medical centers worldwide. In addition to its daily activities and cooperation with infectious diseases specialists, the clinical microbiology laboratory shoulders responsibilities to disseminate information and presents updates and reviews of relevant infectious topics, assesses the antimicrobial susceptibility patterns of newly introduced antimicrobial agents in the country, provides annual susceptibility data useful for empiric treatment and monitors resistance rates trends, as well as alerts about any newly emerging resistance profile or multidrug resistant (MDR) micro-organisms as part of the endeavors to control infections.

The global emergence of antimicrobial resistance constitutes serious human and public health burdens, especially due to limited availability of treatment options. Such resistance entails morbid and mortal threats and challenges in the treatment of patients infected with such pathogens, as well as the infection control tolls associated to these resistant microorganisms [1-4].

This serious situation led, for example, the Infectious Diseases Society of America to raise the alarm and label this threatening situation as "Bad Bugs, No Drugs" [5]. The latter microorganisms span over a wide range of microbial species such as methicillin resistant *Staphylococcus aureus* (MRSA) being healthcare-associated (HA-MRSA) or community-associated (CA-MRSA), vancomycin-intermediate or resistant *S. aureus* (VISA or VRSA), vancomycin-resistant enterococcus (VRE), the multidrug-resistant *Acinetobacter* spp. and *Pseudomonas aeruginosa*, extended-spectrum ß-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* spp., and carbapenem resistant enterobacteriaceae (CRE) [3-4]. Thus, surveillance and revealing the antimicrobial profile as well as monitoring and determining the changing trends of resistance among these pathogens at different periods of time provides essential valuable information for clinical practice, infection control aspects and epidemiologic aspects as cornerstones in the containment of this problem [6-7].

The present study was undertaken to reveal and reflect on antimicrobial resistance among commonly encountered bacteria (pertaining to the ones noted above and others) recovered at the Clinical Microbiology Laboratory (Accredited by The College of American Pathologists) of the American University of Beirut Medical Center (AUBMC) over a decade: 2000-2001 and 2010-2011. In addition, pertinent studies from our institution, are presented.

MATERIALS and METHODS

The analyzed bacteria in this study were recovered from different specimens submitted to the Clinical Microbiology Laboratory of AUBMC. The latter is a major referral center for acute and specialized care in Lebanon. It has 370 beds capacity. The 2010 yearly patient's statistics shows 26,300 admissions, 246,400 out-patient-visits and 47,400 emergency department visits.

To avoid duplication of isolates, the first bacterial isolate or the same species with different antimicrobial pattern or different species recovered from the same patient's specimen were included in this study.

All isolated bacteria were immediately identified based on standard methods [8] and routinely tested against antimicrobial agents based on disk diffusion CLSI guidelines, interpretation and quality control aspects, published for each year [9]. Also based on the latter, an annual brochure entitled *Antimicrobial Susceptibility Patterns of Bacterial Isolates* has been produced and circulated to medical staff.

When indicated or requested, E test (PDM-Epsilometer, AB Biodisk, Solna, Sweden) was used to determine the MICs against certain isolates. Though this decade study primarily provides comparative data on three points in time (2000/1, 2005/6 and 2010/1), the yearly data was also incorporated as ranges from 2000/1 to 2010/1. In addition,

TABLE I
DISTRIBUTION of BACTERIAL ISOLATES vs. SOURCE of SPECIMEN at DIFFERENT YEARS

Bacteria / Year (total)	% of recovered isolates from specimens				
	Respiratory	Blood	Misc	Wound	Urine
***Acinetobacter* spp.**					
2000/1 (n = 257)	51	3	6	29	11
2005/6 (n = 230)	62	9	1	12	16
2010/1 (n = 394)	55	19	4	15	18
P. aeruginosa					
2000/1 (n = 755)	40	2	6	25	27
2005/6 (n = 705)	52	7	1	18	22
2010/1 (n = 940)	45	7	3	20	25
E. coli					
2000/1 (n = 1942)	4	3	7	8	56
2005/6 (n = 2660)	5	9	2	7	77
2010/1 (n = 3811)	5	8	1	6	80
K. pneumoniae					
2000/1 (n = 598)	26	3	7	8	56
2005/6 (n = 590)	23	9	1	7	60
2010/1 (n = 947)	20	11	1	6	62
***Enterococcus* spp.**					
2000/1 (n = 478)	3	16	5	6	70
2005/6 (n = 325)	3	15	2	4	77
2010/1 (n = 604)	4	15	NT	8	73
S. aureus					
2000/1 (n = 378)	15	10	5	67	3
2005/6 (n = 310)	20	12	4	57	7
2010/1 (n = 479)	24	13	6	51	6
S. viridans					
2000/1 (n = 83)	NT	40	26	20	14
2005/6 (n = 118)	NT	45	18	27	10
2010/1 (n = 97)	NT	60	11	18	11
S. pneumoniae					
2000/1 (n = 46)	56	40	2	2	0
2005/6 (n = 57)	53	45	1	1	0
2010/1 (n = 64)	58	38	2	2	0

Misc: Miscellaneous **NT**: not tested

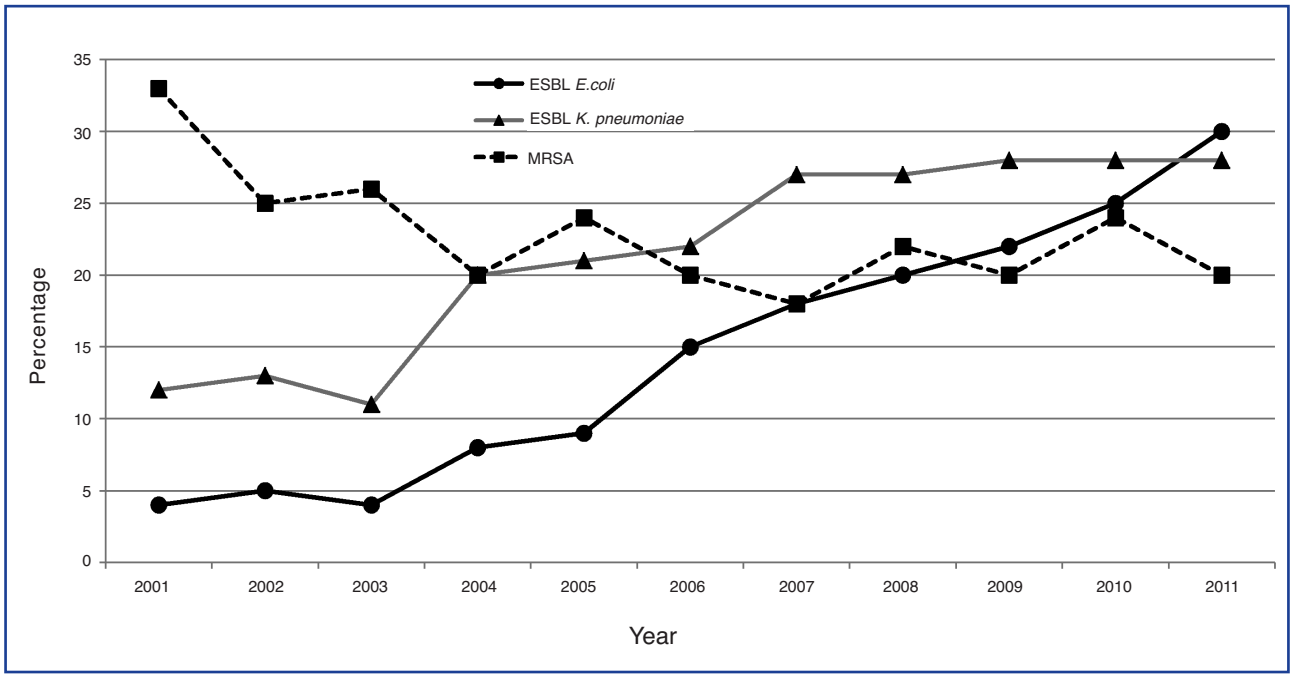

FIGURE 1. Prevalence of ESBL *E. coli* and *K. pneumoniae*, and MRSA over the past years

the present study also addresses and refers to other relevant antimicrobial publications and studies originating or contributed to from our institution during this decade.

Data statistical analysis was carried out using Minitab 15 software system.

The descriptive data was given in mean ± standard deviation (SD). Test and confidence interval for proportions and Fischer's exact test were used for the analytical assessment. The differences were considered to be statistically significant when the *p*-value obtained was less than 0.05.

RESULTS and DISCUSSION

The results are represented in tables, showing the distribution of sources of isolates (Table I) and the antimicrobial susceptibility of pathogens versus years (Tables II-X, and Figure 1). In addition to the results, reviews, researches and studies carried out at AUBMC during this decade in relation to our topic are also noted under each pathogen discussion.

Staphylococcus aureus and coagulase negative staphylococci (CNS)

The rates of MRSA showed remarkable increase between 1971 and 1999 (3% to 38%) [7]. Then it started to decrease from 2000 through 2006 (33% to 20%) to stabilize at 20% in 2010 (range 18-33%) (Table II). So far, no VISA or VRSA were recovered. Fluctuation in susceptibility rates, generally towards the higher and stable trends have been observed against erythromycin (73%-88%), clindamycin

TABLE II
ANTIMICROBIAL SUSCEPTIBILITY of *S. aureus* and COAGULASE NEGATIVE STAPHYLOCOCCI (CNS) vs. YEARS

Bacteria	% (Range) of isolates susceptible against							% MR*
Year (no. tested)	Oxacillin	Erythromycin	Clindamycin	Levofloxacin	Trimeth/Sulf**	Nitrofurantoin	Vancomycin	
S. aureus								
2000/1 (n = 378)	67	84	87	NT	NT	NT	100	33
2005/6 (n = 310)	80▼	80	85	90	94	100▲	100	20
2010/1 (n = 479)	80	84	88	88	96	100♦	100	20
All years (Range)	**(67-82)**	**(73-88)**	**(78-94)**	**(71-100)**	**(88-100)**	**(100-100)**	**(100-100)**	**(18-33)**
CNS								
2000/1 (n = 942)	32	38	69	NT	NT	NT	100	68
2005/6 (n = 1170)	30	30▼	50▼	NT	80	100■	100	70
2010/1 (n = 1665)	20▼	28	55▼	46	56▼	99✖	100	80
All years (Range)	**(20-35)**	**(25-40)**	**(48-69)**	**(42-46)**	**(56-84)**	**(95-100)**	**(100-100)**	**(65-80)**

***MR**: Methicillin resistant ****Trimeth/Sulf**: Trimethoprim/Sulfamethoxazole **NT**: not tested ▼$p < 0.05$
▲17 isolates tested ♦19 isolates tested ■98 isolates tested ✖88 isolates tested

TABLE III
ANTIMICROBIAL SUSCEPTIBILITY of *S. pneumoniae* vs. YEARS

Antimicrobials	% (Range) susceptible in year			All years (Range)
	2000/1 (n = 46)	2005/6 (n = 37)	2010/1 (n = 64)	
Penicillin	35	36	38	(28-40)
Oxacillin	35	36	38	(28-40)
Erythromycin	95	65▼	72	(52-95)
Clindamycin	99	75▼	81	(57-95)
Trimeth/Sulfa**	35	47▼	58▼	(35-60)
Ciprofloxacin/ Levofloxacin	100	100	100	(99-100)*

▼$p < 0.05$ **Trimeth/Sulf: Trimethoprim/Sulfamethoxazole
*Only three isolates were fluoroquinolone-resistant [Ref 19].

(78%-94%), levofloxacin (71%-100%), and trimeth/sulfa (88%-100%). The susceptibility to nitrofurantoin remained uniform (100%). Though not cited in Table II, *S. aureus* susceptibility rates for tetracycline ranged between 82% and 85%, and for gentamicin between 94% and 96%.

The CNS showed increasing rates of resistance to methicillin from 68% in 2000/1 to 80% in 2010/1. Other tested antimicrobials showed also increasing resistant rates except tetracycline and gentamicin whose susceptibility remained stable over this decade (72%-75%; and 64%-65%, respectively).

A persistently high level of resistance (≥ 98%) was observed for penicillin and ampicillin against both *S. aureus* and CNS.

Seven studies emanated during this decade dealing with staphylococci [10-16]. The first four studies addressed rapid detection of MRSA [10], MICs determination of oxacillin and glycopeptide activity against MRSA strains, that didn't reveal any VISA or GISA [11], the *S. aureus* and MRSA nasal carriage rates among university students, found to be 25% and 1.7%, respectively, (almost similar rates found in 2000s and 1990s studies) and the risk factors were associated with male gender, young age, healthcare worker contact, drug injection and asthma [12-13]. The fifth study was a molecular characterization dedicated to investigate the distribution of SCC*mec* types I-V and to detect the PVL and γ-hemolysin (*hlg*) genes in MRSA and MSSA strains. 53% of MRSA harbored the PVL gene, 97% had the *hlg* gene, 86% had the SCC*mec* genes type IVc and 11% had the type III. 20% of MSSA isolates harbored the PVL gene and 97% had the *hlg* gene. The PVL isolates were of type IV. Protein A gene (spa) sequencing identified 48 spa types, and multilocus sequence typing revealed 10 sequence types (STs) among the isolates [14]. The sixth study was the first from Lebanon to demonstrate the incidence of virulent genes showing exfoliative toxin A to be more prevalent than B with virulent determinants being additionally detected in multiple drug-resistant isolates, determine the DNA genetic diversity of *S. aureus* isolates, and perform sequence analysis of the USA 300 clone genetic region designating the arginine catabolic mobile element (ACME) [15]. The seventh study determined the different prevalent CNS species using molecular and conventional methodologies. The predominant species among the 68 CNS clinical isolates revealed *S. epidermidis* (81%), *S. haemolyticus* (3%), *S. lugdunensis* (3%) *S. warneri* (1.5%), *S. saprophyticus* (1.5%), *S. hominis* (1.5%), *S. capitis* (1.5%) and *S. scuiri* (1.5%) [16].

Streptococcus pneumoniae

S. pneumoniae has been showing consistently low susceptibility rates against penicillin (ranging between 28% and 40%) (Table III). It showed decreasing susceptibility trends to erythromycin (95% in 2000/1 to 72% in 2010/1), clindamycin (99% in 2000/1 to 81% in 2010/1), and increasing trends though on the overall lower range against trimeth/sulfa (35% in 2000/1 to 58% in 2010/1). So far, only three strains were resistant to levofloxacin. The oxacillin disk diffusion is a screening test which gives accurate susceptible category results only. However, it does not give actual reflection of the complete resistance or intermediate resistant categories of penicillin, as the latter requires determination by MICs testing. Before 2000, such MICs data for penicillin were presented in an earlier study [17-18].

Five research studies were conducted at our medical center looking at various aspects of *S. pneumoniae* during this 10-year study period [17-21]. The first and second studies determined the antimicrobial susceptibility using MIC testing [17-18]. Among 43 isolates tested, 28% had low susceptibility to penicillin, 58% showed intermediate susceptibility and 14% had high resistance to penicillin. Their susceptibility was uniform (100%) to gatifloxacin, levofloxacin, and amoxicillin/clavulanate, very high to ceftriaxone (93%) and relatively low to cefuroxime (75%), azithromycin (65%), and clindamycin (65%) [18]. Most important to recall are the MIC findings for penicillin, ceftriaxone, cefuroxime, azithromycin (clarithromycin) which were lower than those reported on tested isolates recovered between 1996 and 1998 [17]. The third publication is a report of three *S. pneumoniae* isolates resistant to quinolones recovered between 2006 and 2010 [19]. The fourth publication is a report generated from inter-hospital surveillance program undertaken (between 2005 and 2011)

TABLE IV
ANTIMICROBIAL SUSCEPTIBILITY of *Enterococcus* spp. vs. YEARS

Antimicrobials	% (Range) susceptible in year			All years (Range)
	2000/1 (n = 478)	2005/6 (n = 325)	2010/1 (n = 604)	
Ampicillin	86	95▼	84▼	(84-95)
Erythromycin	12	20▼	14▼	(13-23)
Nitrofurantoin	NT	98▲	99♦	(96-99)
Vancomycin	100	100	99.6	(96.6-100)
Norfoflaxin	NT	70	53▼	(53-77)
Tetracycline	NT	20	18	(18-26)

▼$p < 0.05$ **NT**: not tested ▲146 isolates tested ♦270 isolates tested

to evaluate the serotype prevalence and antibiotic susceptibility of 240 clinical isolates of *S. pneumoniae* involved in invasive pneumococcal disease (IPD) were determined [20]. Most infection involved patients > 60 years (33%) and ≤ 2 years (23%). Most samples were collected from the blood (78%). Mortality was 12%. Pneumonia was the most prevalent presentation (48%). Susceptibility of the isolates showed 56% to penicillin G, 89% to ceftriaxone, 73% to erythromycin and 100% to levofloxacin. The most prevalent serotypes/serogroups were: 19F (12%), 6 (8%), 3 (7.5%), 1 (7%), 14 (7%), 19A (5%). Vaccine coverage was 40% for PCV7, 53% for PCV10, and 66% for PCV13 when all age groups were considered but was higher for children under 5 years of age. The study underscores the importance of conducting surveillance studies to identify epidemiologic characteristics, antibiotic susceptibility, and serotype prevalence of IPD in order to raise awareness about vaccination strategies that help decrease the disease burden at the national level [20]. The last study dealt with the molecular characterization of macrolide resistance, and the prevalence of circulating serotypes of *S. pneumoniae* isolates between 2008 and 2010 [21]. Forty-four isolates were studied, among which 79% were resistant to penicillin and 41% to ceftriaxone. *erm* (B) gene (encoding methylase) was present in 36% of these resistant isolates, *mef* gene (encoding macrolide efflux pump protein) in 18%, both *erm* (B) and *mef* genes in 32% and no *erm* (B) and *mef* genes in 14%. Seven different capsular serotypes 2, 9V/9A, 12F, 14, 19A, 19F and 23A, were detected, with 19F being the most prevalent and all with exception of serotype 2 being invasive. Invasive serotypes 14 and 19F harbored both *erm* (B) and *mef* genes. Among 21 erythromycin susceptible isolates, nine different serotypes were detected with 4 isolates belonging to serotype 5, 3 to serotype 9V/9A, 2 to serotype 6A/B/C, 3 to serotype 2, and 1 each to serotypes 15B/15C, 4 , 21, 38, and 35 [21].

TABLE V
ANTIMICROBIAL SUSCEPTIBILITY TEST RESULTS
of *S. viridans, S. pyogenes* and *S. agalactiae* vs. *YEARS*

Bacteria Year (no. tested)	% (Range) susceptible against Penicillin	Erythromycin	Clindamycin
S. viridans			
2000/1 (n = 83)	95	77	83
2005/6 (n = 118)	88▼	65▼	75
2010/1 (n = 97)*	91	72	81
All years (Range)	**(85-96)**	**(60-83)**	**(73-92)**
S. pyogenes			
2000/1 (n = 84)	100	100	95
2005/6 (n = 135)	100	90▼	92
2010/1 (n = 273)	100	88	88▼
All years (Range)	**(100-100)**	**(85-100)**	**(92-98)**
S. agalactiae			
2000/1 (n = 67)	100	95	95
2005/6 (n = 130)	100	97	97
2010/1 (n = 155)	100	97	98
All years (Range)	**(100-100)**	**(80-97)**	**(80-98)**

▼$p < 0.05$ *Ciprofloxacin/Levofloxacin = (96%)

Enterococcus

Enterococcus spp. showed decreased susceptibility to ampicillin (95% to 84%) and norfloxacin (95% to 84%), persistent very low susceptibility to erythromycin (13%-23%) and tetracycline (18%-26%), and very high susceptibility to vancomycin, ranging between 99.6% and 100% (Table IV). Only seven vancomycin resistant enterococci (VRE) strains were detected through routine testing during this period.

Only one study was done during the past decade dealing with the *Enterococcus* spp. in 2002, revealing the species and susceptibility of this pathogen [22]. Among 53 isolates, the frequency of species were *E. faecalis* (72.5%), *E. faecium* (22.9%), *E. avium* (3.3%) and *E. gallinarum* (1.3%). Differences in resistance rates were noted between *E. faecalis* and E. *faecium*. One isolate of *E. gallinarum* showed intermediate resistance (MIC 16 µg/ml) to vancomycin; the remaining were all susceptible. High level aminoglycoside (HLA) resistance was detected among *E. faecalis* and *E. faecium* being, respectively, 19% & 39% against gentamicin and 36% & 26% against streptomycin [22].

Streptococcus viridans group

The viridans streptococci have been showing fluctuating but decreasing trends of susceptibility to penicillin (96% to 85%), erythromycin (83% to 60%), and clindamycin (92% to 73%), (Table V).

Streptococcus pyogenes (group A strep or GAS)

The groups and the rate range distribution of recovered ß-hemolytic streptococci over the decade were group A (38%-60%), group B (30%-54%), group C (1%-8%), group G (4%-12%). *S. pyogenes* showed decreasing susceptibility to erythromycin (from 100% to 88%) and clindamycin (from 95% to 88%), but maintained uniform susceptibility to penicillin (Table V).

Research on GAS during this 10-year study period was limited to studying the *emm* typing, antibiotic resistance and PFGE analysis [23]. Among 103 S. *pyogenes* isolates analyzed, 33 *emm* types and subtypes were detected. The most prevalent were: *emm*1 (12.6%), *emm*22 (8.7%), *emm*28 (7.7%), *emm*88 (7.7%) and *emm*4 (6.8%). All isolates were susceptible to penicillin G and vancomycin, while 10% were resistant to erythromycin and 3% were resistant to erythromycin and clindamycin, showing the macrolide-lincosamide-streptogramin B phenotype. This study will serve as a basis for information for long-term evolutionary and epidemiological studies of local *S. pyogenes* recovered not only in Lebanon, but also in neighboring countries [23].

Streptococcus agalactiae (group B strep or GBS)

S. agalactiae also maintained its stable (100%) susceptibility to penicillin (Table V). Their susceptibility to ery-

thromycin and clindamycin were very high (95%-98%) during the years 2000, 2005, and 2010. In between these years, however, fluctuation (80% and 98%) in susceptibility was observed.

Two research studies were conducted at our medical center on *S. agalactiae* during this 10-year study period [24-25]. The first study determined high prenatal and neonatal GBS colonization rates [24]. Overall, 17.7% of mothers and 7.3% of newborns tested positive for GBS. Serotype 5 (24.1%) was the most prevalent followed by serotype 1a (15.0%), 3 (14.4%), 2 (11.8%) and 1b (7.5%). These results could provide basis for the institution of a national policy for universal maternal GBS screening to reduce neonatal morbidity and mortality [24]. The second was a molecular study on 76 GBS whereby PCR analysis of virulence factors encoding genes revealed *cylE* gene presence in 99% of the isolates, *lmb* in 96%, *scpB* in 94.7%, *rib* in 33%, and *bca* in 56.5% [25]. All GBS isolates were susceptible to penicillin G, cefepime, ceftriaxone, and levofloxacin. Resistance was observed against chloramphenicol (l4%), clindamycin (11.8%), erythromycin (15.8%), and tetracycline (86.8%) [25].

E. coli and *Klebsiella* spp.

Both *E. coli* and *Klebsiella* spp. are among the most commonly recovered Gram-negative bacteria species from clinical specimens. In our present study, the *E. coli* isolates have been showing increasing resistance against most cephalosporins, fluoroquinolones, and gentamicin. The susceptibility to amoxi/clav remains variable and that of ampicillin, cephalothin and trimeth/sulfa remains constantly low (Table VI). A relatively high and stable susceptibility are maintained for amikacin, cefoxitin, pipera/tazo, and nitrofurantoin. Cefoxitin is a surrogate marker for MDR status. Cefepime testing started in 2006 and showed a susceptibility rate ranging between 83% and 95%, where the latter rate was first noted at the initial testing year (Table VI). Two serious and worrisome observations have been noted: the remarkable increasing encounter of ESBL producing isolates (ranging from 4% in 2000 to 30% in 2011) (Figure 1), and the threatening compromise on the uniform susceptibility to imipenem by the recent emergence of some carbapenem resistant *E. coli* strains, first imported from medical tourists.

Klebsiella spp. isolates, same as *E. coli*, show increasing resistance to cefixime, ciprofloxacin, and trimeth/sulfa (Table VII). Susceptibility to cephalothin was maintained at lower rate 60s % (not noted in the Table). Susceptibilities, toward increasing trends, were observed against amox/clav (66% to 81%), ceftazidime (71% to 79%), pipera/tazo (78% to 93%), and amikacin (83% to 98%). Consistently stable susceptibility were observed against aztreonam (71%-74%), cefoxitin (94%-96%), cefuroxime (69%-75%), cefotaxime (70%-75%), gentamicin (81%-83%) and nitrofurantoin (around 58%). Cefepime testing started in 2006 and showed a susceptibility rate ranging between 80% and 88%. Similar to *E. coli* problems, the two serious and worrisome observations have been noted for the remarkable increasing encounter of ESBL producing *K. pneumoniae* (ranging from 12% in 2000 to 28% in 2011) (Figure 1), and the threatening compromise on the

TABLE VI
ANTIMICROBIAL SUSCEPTIBILITY of *E. coli* vs. YEARS

Antimicrobials	% (Range) susceptible in year			All years (Range)
	2000/1 (n = 1942)	2005/6 (n = 2660)	2010/1 (n = 3811)	
Ampicillin	33	28	27	(25-33)
Amoxicillin/ Clavulanate	57	55	77▼	(55-75)
Aztreonam	91	80▼	71▼	(71-93)
Cefepime	NT	NT	83	(83-95)
Cefexime	87	82▼	70▼	(70-87)
Cefoxitin	93	91	92	(89-95)
Cefuroxime	85	75▼	65▼	(64-85)
Cefotaxime	92	80▼	67▼	(67-93)
Ceftazidime	92	82▼	76▼	(76-92)
Piperacillin/ Tazobactam	91	86▼	94▼	(86-97)
Imipenem	100	100	99.9	(99.9-100)
Ciprofloxacin	73	60▼	53▼	(53-73)
Amikacin	97	96	99	(96-99)
Gentamicin	86	76▼	73▼	(72-86)
Trimethoprim/ Sulfamethoxazole	51	42▼	45▼	(40-51)
Nitrofurantoin	NT	95*	98▲	(94-97)
ESBL	4	15	30	(4-30)

▼$p < 0.05$ **NT**: not tested *1884 isolates tested ▲2761 isolates tested. Carbapenem resistance is associated with imipenem, ertapenem, and meropenem.

TABLE VII
ANTIMICROBIAL SUSCEPTIBILITY of *K. pneumonia* vs. YEARS

Antimicrobials	% (Range) susceptible in year			All years (Range)
	2000/1 (n = 598)	2005/6 (n = 590)	2010/1 (n = 947)	
Amoxicillin/ Clavulanate	66	65	81▼	(62-79)
Aztreonam	71	76	74	(70-76)
Cefepime	NT	NT	88	(80-88)
Cefexime	86	80▼	76▼	(63-88)
Cefoxitin	94	95	96	(93-97)
Cefuroxime	69	75▼	70▼	(68-81)
Cefotaxime	70	75▼	71▼	(70-83)
Ceftazidime	71	75▼	79▼	(71-84)
Piperacillin/ Tazobactam	78	78	93▼	(72-93)
Imipenem	100	100	99.3	(98-100)
Ciprofloxacin	86	83▼	74▼	(72-90)
Amikacin	83	95▼	98▼	(83-99)
Gentamicin	83	82	81	(75-91)
Trimethoprim/ Sulfamethoxazole	73	68▼	58▼	(58-78)
Nitrofurantoin	NT	58*	58▲	(47-58)
ESBL	12	22	28	(11-28)

▼$p < 0.05$ **NT**: not tested *278 isolates tested ▲410 isolates tested Carbapenem resistance is associated with imipenem, ertapenem, and meropenem.

TABLE VIII
ANTIMICROBIAL SUSCEPTIBILITY of *Acinetobacter* spp vs. YEARS

Antimicrobials	% (Range) susceptible in year			All years (Range)
	2000/1 (n = 257)	2005/6 (n = 230)	2010/1 (n = 394)	
Aztreonam	4	3	7	(3-8)
Cefepime	NT	NT	21	(20-30)
Ceftazidime	17	25▼	18▼	(16-45)
Piperacillin/ Tazobactam	18	25	20▼	(16-40)
Imipenem	99	80▼	30▼	(30-100)
Ciprofloxacin	21	15▼	17	(13-45)
Amikacin	28	20▼	24	(20-55)
Gentamicin	20	17	34▼	(17-50)
Trimethoprim/ Sulfamethoxazole	41	20▼	19	(15-65)

▼$p < 0.05$ **NT**: not tested

uniform susceptibility to imipenem (Table VII) by the recent emergence of some carbapenem resistant strains, imported to Lebanon by medical tourists.

Due to the surging incidence of resistant *Enterobacteriaceae*, many research studies were conducted to try to predict, survey, overcome, manage and deal with the problems and challenges created by the ESBLs- and carbapenem-resistant *E. coli* and *K. pneumoniae* during this 10-year study period.

Ten studies were published elaborating, among others, the susceptibility patterns, risk factors, clinical and microbiologic profiles, and molecular characterization of the ESBL-producing *E. coli* and *K. pneumoniae* [26-35]. A couple of these are highlighted. A study was conducted dealing with tigecycline since it was introduced as a salvage therapy for MDR *Enterobacteriaceae*, *Acinetobacter* spp. and others. The percentage of susceptible ESBL-producing strains among 150 *E. coli* and 100 *K. pneumoniae* were 100%, and 81% to tigecycline. The *Acinetobacter* spp. susceptibility to tigecycline was 98% (also further noted under *Acinetobacter* spp. below) [29]. Another study addressed the prevalence of different ESBLs *bla* genes among 50 *E. coli* isolates were CTXM 96%, TEM 57%, SHV 67%, and among 50 *K. pneumoniae* isolates were CTXM 40%, TEM 82%, and SHV 84% [33]. Sequence analysis of 16 selected isolates identified the *bla*CTX-M-15, *bla*TEM-1, *bla*OXA-1 and six *bla*SHV genes, as well as the gene encoding the quinolone modifying enzyme AAC(6')-Ib-cr [33]. Moreover and because the CTX-M type enzymes have become the most prevalent ESBLs, and CTX-M-15 ESBLs encoding genes being the most widespread worldwide among *Enterobacteriaceae*, a review about its spread in the Middle Eastern Region was recently undertaken [35].

The attention on carbapenem resistant *Enterobacteriaceae* (CRE) especially among *E. coli* and *K. pneumoniae* constituted a major threat since the global and local recovery of these organisms in 2008. Six studies were published from our institution on this problem [36-41]. A couple of theses studies revealed the involvement of a plasmid encoded ß-lactamase (*bla*-OXA-48) gene being responsible for resistance to imipenem in *K. pneumoniae* [36-37]. In addition to this gene, and starting 2010, increasing numbers of CRE has been noticed now harboring the very threatening *bla* NDM-1 gene producing the novel New Delhi Metallo-ß-lactamase. The latter gene was detected the first time in Lebanon, in *E. coli* and *K. pneumoniae* isolates that both were recovered from Iraqi patients coming for medical care at AUBMC [38]. Such findings warranted assessing the MICs of doripenem, meropenem and imipenem against MDR *E. coli* and *K. pneumoniae* revealing resistance rates of 1%, 1% and 4%, respectively, for the former isolates, and 1.8%, 1.8% and 8.8%, respectively, for the latter [39]. The mechanisms and genes responsible for the carbapem resistance in *K. pneumoniae* and *E. coli* among our isolates were addressed in a molecular study revealing the presence of different ß-lactamases gene profiles including: $bla_{\text{-OXA-1}}$, $bla_{\text{-CTXM-15}}$, $bla_{\text{-TEM-1}}$, $bla_{\text{-CMY-2}}$, $bla_{\text{-OXA-48}}$ and NDM-1 genes in both; in addition, the *K. pneumoniae* isolates were found to lack outer membrane porins (OmpC and OmpF) encoding genes while *E. coli* harbored these porin genes [40]. Such critical resistance pathogens mandated an updated highlight to the medical and paramedical community on the local and regional epidemiology of carbapenem resistance in the Middle East, the mechanisms involved, screening and detection methods, as well as their treatment and control aspects [41].

Acinetobacter spp. and *Pseudomonas aeruginosa*

These pathogens are among the most, if not, the most threatening nosocomial MDR organisms. Even, it is not unusual to encounter nosocomial outbreaks and/or pan resistant strains among them, and the latter has been detected since 1998 [7]. The overall susceptibility of *Acinetobacter* spp. to the vast majority of antimicrobial agents is very low (Table VIII). Most notably, is the remarkable resistance encountered against imipenem and other car-

TABLE IX
ANTIMICROBIAL SUSCEPTIBILITY of *P. aeruginosa* vs.YEARS

Antimicrobials	% (Range) susceptible in year			All years (Range)
	2000/1 (n = 755)	2005/6 (n = 705)	2010/1 (n = 940)	
Aztreonam	67	75▼	90▼	(67-90)
Cefepime	NT	NT	90	(85-94)
Ceftazidime	81	80	88▼	(80-92)
Piperacillin/ Tazobactam	80	78	84▼	(76-87)
Imipenem	85	78▼	80	(75-86)
Ciprofloxacin	78	77	83▼	(77-88)
Amikacin	84	81	91▼	(81-96)
Gentamicin	77	76	89▼	(76-86)

▼$p < 0.05$ **NT**: not tested

bapenems (resistance to imipenem increased from 1% in 2000/1 to 70% in 2010/1). The MIC_{90} for 72 MDR *A. baumannii* isolated in 2010 against doripenem, meropenem and imipenem, were ≥ 32 mg/L for each, and the susceptibility rates were 38.9%, 36.1% and 16.7%, respectively [39]. Tigecycline being one of the few remaining effective drugs, 98% of the *Acinetobacter* spp. analyzed between 2006-2007 were susceptible [29], and this rate is maintained till todate.

With respect to *P. aeruginosa*, their susceptibilities to the different antimicrobial agents show fluctuations, generally between 70s and 80s percent, tending lately to be towards the higher levels of susceptibility (Table IX). The MIC_{90s} for 40 MDR *P. aeruginosa* against doripenem, meropenem and imipenem, were > 32 mg/L for each, and the susceptibility rates were 65%, 47.5%, and 27.5%, respectively [39]. The most virulent strains were of the genotype 1 and were the most sensitive [42].

Haemophilus influenzae and *Moraxella catarrhalis*

Both remain important etiologies for respiratory tract infections. *H. influenzae* showed fluctuating susceptibility to ampicillin (69% to 82%, which parallel that of ß-lactamase producing), stable high susceptibility to amox/clav (95% to 100%), cefuroxime, macrolides (azithromycin/clarithromycin 97% to 100%) and fluoroquinolones (97% to 100%), and moderate susceptibility to trimeth/sulfa (65%-76%) (Table X). ß-lactamase negative ampicillin resistant (BLNAR) strains were rarely detected over the decade (1-2 strains per year detected in four of the last ten years). Susceptibility to ceftriaxone remained uniform. *H. influenzae* serotype b detection showed fluctuation in pre-valence (40%-86%) tending to be on the increase occurrence compared to not type b.

Concerning *M. catarrahalis*, the rate of ß-lactamase producing strains over the study decade was ≥ 97%. However, over the decade, very high susceptibility was observed against amox/clav (100%), cefuroxime (100%), fluoroquinolones (100%) and macrolides (95%-100%), and the susceptibility against trimeth/sulfa was fluctuating (80%-100%).

Only one study was conducted at our medical center over the decade in which the sensitivity of *H. influenzae* and *M. catarrahalis* was analyzed [18]. Both of the organisms were ≥ 95% susceptibility to gatifloxacin, levofloxacin, ceftriaxone, amoxi/clav, cefuroxime and azithromycin. Ampicillin susceptibility against *H. influenzae* was 78%. ß-lactamase production in *M. catarrhalis* was ≥ 97% [18].

Salmonella

The rates of recovery among different *Salmonella* spp. over the decade varied in ranges possibly due to different source origin of the isolates partially attributed to tourists from the African continent especially during the summer. The most frequent types of *Salmonella* spp. and their range of recovery rates were: *S. enteritidis* (18%-44%), *S. typhimurium* (12%-34%), *S. typhi* (0%-23%), *Salmonella* group C (7%-23%), *S. paratyphi* B (0%-9%), *Salmonella* group B (0%-7%), *S. paratyphi* A (0%-1%), and the nontypable *Salmonella* spp. with our available antisera (4%-18%).

TABLE X
ANTIMICROBIAL SUSCEPTIBILITY of *H. influenzae* vs. YEARS

Antimicrobials	% (Range) susceptible in year			All years (Range)
	2000/1 (n = 95)	2005/6 (n = 106)	2010/1 (n = 102)	
Ampicillin	71	88▼	85	(69-82)
Amoxicillin/ Clavulanate	100	100	100	(95-100)
Cefuroxime	100	100	100	(100-100)
Levofloxacin	NT	99	97	(97-100)
Trimethoprim/ Sulfamethoxazole	70	70	67	(65-76)
Azithromycin/ Clarithromycin	NT	97	100	(97-100)
Positive ß-lactamase	29%	12%	15%	(12-31)

▼$p < 0.05$ **NT**: not tested

S. typhi maintained the uniform susceptibility to ampicillin, cefotaxime, ceftazidime, ciprofloxacin and trimeth/sulfa till 2004. Thereafter, few resistant strains started to emerge with variable susceptibility to ampicillin (65%-100%) and trimeth/sulfa (43%-100%). Fortunately and so far, no *S. typhi* resistant strains to third generation cephalosporins or fluoroquinolones were detected.

The situation of non typhi *Salmonella* spp. is different from that of *S. typhi* in showing higher resistant rates to antimicrobial agents. Fluctuation in susceptibility to ampicillin has been ranging from 65% to 90% and against trimeth/sulfa from 82% to 98% over the decade study. Compromised susceptibility to third generation cephalosporins and fluoroquinolones has been detected since 2005, revealing the following susceptibility ranges: cefotaxime (88%-100%), ceftazidime (87%-100%), and ciprofloxacin (91%-100%). Nalidixic acid resistance among *Salmonella* spp. tested since 2008 ranged between 25%-57%. The importance of detecting nalidixic acid resistance alerts about the possibility of clinical failure in using the fluoroquinolones for the treatment of infection with such pathogens.

Two case report studies were conducted during this decade covering the *Salmonella* spp. [43-44]. The first reported on genital ulceration in two sisters associated with typhoid fever [43]. The second reported a case of *S. enteritidis* in a patient with systemic lupus erythematosus causing septic arthritis to highlight on the prevalence of such organisms in immunodeficient patients [44].

Shigella spp.

The rates of recovery among different *Shigella* spp. over the decade also varied in ranges possibly due to different source origin of the isolates as could be attributed to travel and tourism.

The most common types of *Shigella* spp. and their range of recovery rates were: *S. sonnei* (group D) (55%-

87%), *S. flexneri* (group B) (5%-46%), S. *dysenteriae* (group A) (0%-33%), *S. boydii* (group C) (0%-20%), and the nontypable *Shigella* spp. with our available antisera (0%-5%). *Shigella* spp. showed a fluctuation in the susceptibility to ampicillin (ranging from 35% to 84%) and trimeth/sulfa (ranging from 9% to 35%), remained uniformly susceptible to ciprofloxacin and showing a wide range of susceptibility to cefotaxime (70% to 100%) and ceftazidime (80% to 100%).

Resistant *Shigella* spp. to third generation cephalosporins were first detected in Lebanon in 2005, subsequently increasing and revealing ESBLs strains producing CTXM-15 [45-46].

Campylobacter spp. & *Helicobacter pylori*

An earlier study in the late 1998 reported on the prevalence, antimicrobial susceptibility patterns and the molecular profile of *Campylobacter* isolates from humans and poultry [47]. The low prevalence detected in human stool specimens (2 of 281 tested, 0.7%) excluded justification to look for such pathogens in routine stool investigation, and left it under special requests when suspected. This explains the lack of substantial antimicrobial data on such pathogens.

Two researches conducted at our medical center on *H. pylori* during this 10-year study period included two studies [48-49]. The first study determined the resistance prevalence of 54 *H. pylori* isolates showing: 29.5% resistance to metronidazole, 4% to clarithromycin, and 2% to tetracycline [48]. All isolates were susceptible to amoxicillin. The prevalence of metronidazole resistance in our study was lower than that from other parts of the Middle East and the developing world. The second study reported the efficacy of two rabeprazole/gatifloxacin-based triple therapies for *H. pylori* infection [49]. *H. pylori* strains were susceptible to amoxicillin and gatifloxacin in vitro. It was concluded that a 7-day regimen of gatifloxacin-rabeprazole-amoxicillin is effective eradication therapy for *H. pylori* [49].

Limitations

Though this study originates from a major referral medical center in Lebanon, it by no way claims to reflect the overall resistance profile in this country. Other limitations can include: the referral bias, possible overestimation of certain resistance rates due to possible inclusion of duplicate isolates and the use of changing cutoff antimicrobial susceptible breakpoints *vs* years based on CLSI annual guidelines.

CONCLUSION

Antimicrobial-resistant organisms are almost always associated with increased attributable mortality, prolonged hospital stays and excess costs [50]. Thus, knowledge about the local antimicrobial susceptibility patterns is needed to guide empirical therapy for the various infections encountered in the community as well as hospital setting, especially the life-threatening ones. Bacterial isolates from Lebanon, herein represented by data from AUBMC, show high rates in some and fluctuating rates in others of antimicrobial resistance. In order to help control infection, guide empirical antibiotic therapy and implement a policy of antibiotic use to curb and prevent any continuation of misuse and the alarming rising rates in antimicrobial resistance, several intervention measures should be disseminated, promoted, implemented and maintained. These include providing a guide to empirical antibiotic therapy, implementation of antibiotic usage policy, monitoring and surveillance of antibiotic resistance, hand hygiene and other infection control measures, and antibiotic stewardship to ensure appropriate use of antimicrobial agents.

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