Original Article

Antibacterial Resistance in Lower Respiratory Tract Bacterial Pathogens: A Multicenter Analysis from Turkey

Aylin Uskudar Guclu[1], Aylin Altay Kocak[1], Mehtap Akcil Ok[2], Bulent Tutluoglu[3], Ahmet Basustaoglu[1], Respiratory Study Group

[1] Department of Medical Microbiology, Faculty of Medicine, Baskent University, Ankara, Turkey
[2] Department of Nutrition and Dietetics, Faculty of Health Sciences, Baskent University, Ankara, Turkey
[3] Department of Chest Diseases, Acibadem International Hospital, Istanbul, Turkey

Abstract

Introduction: This study aimed to evaluate the etiology of lower respiratory tract infections (LRTIs) and their antibiotic resistance.
Methodology: Bacterial culture results of LRT samples from 17 hospitals between 2016-2019 were included in the study. All isolates were identified and AST were performed by automated microbiology systems. AST was performed according to EUCAST.
Results: Non-duplicate 30,051 (26,890 HA and 3156 CA) isolates detected as causative pathogen. LRTIs are caused by 85.1% Gram-negative bacterial pathogens and 14.9% Gram-positive. The most common isolates among HA pathogens were *Acinetobacter spp.* (27.4%), *P. aeruginosa* (22.2%), *K. pneumoniae* (17.9%); among CA pathogen *S. pneumoniae* (19.9%), *P. aeruginosa* (18.9%), *H. influenzae* (14.6%). ESBL rate was 62.5% in *K. penumoniae*; 53.1% in *E.coli*; 19.1% in *Klebsiella spp;* 13.9% in *Enterobacter spp.;* 8.6% in *Proteus spp.*; 6.3% in *Citrobacter spp.*; and 4.3% in *Serratia spp.* Resistance rates to carbapenems and colistin were 92.8% and 12.8% in *A baumannii,* 39.8% and 7.5% in *P. aeruginosa,* 47.3% and 18.5% in *K. penumoniae.* Among staphylococci, 27.3% of *S. aureus* and 82.4% of CoNS were methicillin resistant. 7.6% of *E.faecium* and 0.9% of *E. faecalis* were vancomycin resistant. Linezolid resistant *S. aureus*, CoNS, *E. faecalis* and *E. faecium* rates were 0.3%, 2.9%, 0.0% and 4.6%. Inducible clindamycin resistant rate was 17.2% in S. aureus 38.2% in CoNS. Non-susceptible *S. pneumoniae* isolate rate to penicillin was 37.0%. 6.5% of *S. maltophilia* and 4.4% of *B. cepacia* isolates were resistant to trimethoprim/sulfamethoxazole.
Conclusions: Antibiotic resistance was mainly observed among *A. baumannii* and *K. pneumoniae* and continuous surveillance of antimicrobial resistance patterns in the management of LRTIs is important.

Key words: Lower respiratory tract infection; antibacterial resistance; bacterial etiology.

Introduction

Lower respiratory tract infections (LRTIs) account for more than the total global burden of other diseases, even by excluding the diseases as persistent and pervasive respiratory tuberculosis and pneumonias in patients with HIV/AIDS [1]. LRTIs are the main cause of morbidity and mortality worldwide [2] and account for the most frequent indication for antibiotic prescriptions [3]. It was also reported that unnecessary antibiotic usage and practicing self-medication with antibiotics can lead to antibiotic resistance [4]. Antimicrobial therapy of RTI is usually empirical [5] that physicians often prescribe antibiotics to satisfy a patient or to prevent a worsening of symptoms or to prevent secondary infections [6]. As a result of this uncontrolled usage of broad-spectrum antibiotic has led to the emergence of antibiotic resistance worldwide.

Increased prevalence of resistance among both community-acquired (CA) and hospital-acquired (HA) pathogens is a global concern at regional, national and international levels. It is not possible to eliminate antimicrobial resistance completely, but it is possible to control resistance development with rational antibiotic usage policies [7]. Strengthening the knowledge through education, research and surveillance of antimicrobial resistance is one of the five objectives of the global action plan to reduce antimicrobial resistance [8]. Although there are many surveillance studies on the prevalence of antibiotic resistance, only a few are specific for the origin of infection such as LRTIs. Some major respiratory tract infections (RTI) surveillance studies [5,9,10] provided valuable data on global antimicrobial resistance and they demonstrated that resistance patterns vary significantly from country to

country and between continents [11]. Therefore, the results of multicenter studies are very important for fighting against resistant microorganisms.

In this study, we aimed to evaluate the epidemiology of lower respiratory tract (LRT) pathogens and determine the prevalence of resistance rates of antibiotics including recommended antibiotics by WHO, the Expert Committee on the Selection and Use of Essential Medicines [12] and some resistance mechanisms- including ESBL, inducible clindamycin resistance and as well as focus on resistance (such as; carbapenem, methicillin, vancomycin and high-level gentamicin) of these isolates.

Methodology

Study design

This study was approved by Baskent University Institutional Review Board (Project no: KA17/330). The study included sputum, tracheal aspirate (TA), bronchoalveolar lavage (BAL), bronchial washing/brushing and pleural fluid samples received at 17 centers from 11 cities (Ankara, Adana, Balikesir, Gaziantep, İstanbul, Izmir, Kayseri, Kocaeli, Manisa, Tekirdag, Zonguldak) (these cities represent approximately 40% of the country population) between January 2016 - January 2019. All participating centers use BD Phoenix or Vitek Biomerieux automated test systems, members of an external quality control program supervised by the Ministry of Health.

Identification of isolates

A total of non-duplicated 30,051 bacteria were included in the study which are isolated from 17 different centers as lower respiratory pathogens. The quality of the sputum specimens was evaluated by Gram's staining method. Any growth was considered significant for sterile pleural fluids and pleural aspirate cultures. Colony count of $\geq 10^4$ CFU/ml of quantitative cultures of TA and BAL specimens were considered as significant [13].

All isolates were identified by an automated identification and susceptibility testing system for method association (BD Phoenix™ or Vitek Biomerieux). *Mycobacterium tuberculosis*, anaerobic species and some atypical pathogens (such as *Mycoplasma pneumoniae, Legionella pneumophila, Chlamydia pneumoniae and Coxiella burnetii*) were not included the study.

Susceptibility testing

Antimicrobial susceptibility testing was (AST) performed by automated systems and were evaluated according to the EUCAST standards (2016). AST for *Haemophilus influenzae*, *Moraxella catarrhalis,* and *Streptococcus pneumoniae,* were performed by disk diffusion method and MIC detections were performed by E-test according to EUCAST standards. Colistin susceptibility test results were accepted only if they were performed by broth micro dilution method. ESBL detections were performed by using the differences in growth response to certain second or third generation cephalosporins, with or without clavulanic acid.

Statistical Analysis

Statistical analysis was performed by IBM SPSS version 25.0. (SPSS Inc., Chicago IL, USA) to evaluate

Table 1. Overview of Total, Hospital- and Community-acquired Isolate Distribution

	Total		HA*		CA**		
	N	%	N	%	N	%	*p*
E. coli	2376	7.9	2148	8.0	228	7.2	*0.767*
K. pneumoniae	5140	17.1	4822	17.9	318	10.1	***0.0005***
Enterobacter spp.	711	2.4	649	2.4	62	2.0	*0.8108*
Enterobacterales	1338	4.5	1234	4.6	104	3.3	*0.7129*
A. baumannii	7533	25.1	7364	27.4	169	5.3	*< 0.001*
P. aeruginosa	6575	21.9	5976	22.2	599	18.9	*0.0703*
Other NF***	903	3.0	809	3.0	94	3.0	*0.7494*
S. aureus	2326	7.7	2012	7.5	314	9.9	*0.1739*
CoNS****	223	0.7	206	0.8	17	0.5	*0.1931*
S. pneumoniae	1317	4.4	688	2.6	629	19.9	*< 0.001*
Streptococcus spp.	158	0.5	115	0.4	43	1.4	*0.6824*
Enterococcus spp.	349	1.2	344	1.3	5	0.2	*0.0745*
Other	14	0.0	7	0.0	7	0.2	*-*
H. influenzae	882	2.9	421	1.6	461	14.6	*< 0.001*
M.catarrhalis	206	0.7	95	0.4	111	3.5	*0.286*
Total	**30,051**		**26,890**		**3161**		

*HA: *hospital-acquired;* **CA:*community-acquired;* ****NF:Non-Fermenters; ****CoNS: Coagulase negative staphylococci.

the data obtained from the centers. The difference between the ratios was evaluated by using *Pearson's* chi-square test and *p* < 0.05 was considered as significant.

Results

From 17 different centers, a total of non-duplicated 30,051 bacteria from lower respiratory tract samples were included in the study. Among hospital acquired (HA) pathogens 87.5% were Gram-negative while 12.5% was Gram-positives; among community acquired (CA) pathogens, these rates were 64.7% and 35.3% respectively. In total LRTIs are caused by 85.1% Gram-negative bacterial pathogens and 14.9% Gram-positives (X^2 = 1156.25; *p* < 0.0001). In total 63.9% of causative bacterial pathogens were isolated from TA, 28.7% from sputum, 6.8% from BAL and 0.6% from other respiratory specimens including pleural fluid.

Among HA pathogens, the most common isolate was *Acinetobacter* spp. (27.4%) followed by *Pseudomonas aeruginosa* (22.2%), *Klebsiella pneumoniae (17.9%)*, *Escherichia coli* (8.0%) and *Staphylococcus aureus* (7.5%). Within the CA isolates the most prevalent was *S. pneumoniae* (19.9%) followed by *P. aeruginosa* (18.9%), *H. influenzae* (14.6%), *K. pneumoniae (10.1%)* and *S. aureus* (9.9%). *K. pneumoniae* (*p* = 0.0005) and *Acinetobacter baumannii* (*p* < 0.0001) isolates were detected more in HA LRTIs than CA; while *S. pneumoniae* (*p* < 0.0001) and *H. influenzae* (*p* < 0.0001) isolates are detected more in CA LRTI cases than HA (Table 1). Other *Enterobacterales* members included various species of *Klebsiella*, *Serratia*, *Proteus*, *Citrobacter*, *Providencia*, *Raoultella* genus. Other non-fermenters included

Pseudomonas fluorescens, Pseudomonas putida; Stenotrophomonas maltophilia, Acinetobacter lwoffii, Achromobacter spp. *Chryseobacterium* spp. *Sphingomonas* spp., *Burkholderia cepacia,* and rare isolates such as *Delftia acidovorans, Brevundimonas diminuta, Elizabethkingia meningoseptica.*

Among total isolates of LRTIs, *A. baumannii* was the most common isolate *(*25.1%), followed by *P. aeruginosa* (21.9%) and *K. pneumoniae (*17.1%). The most effective antibiotic against *A. baumannii* isolates was colistin with 12.9% resistance rate. Resistance rates to imipenem and meropenem were 92.8% and 93.1% in *A. baumannii* and 39.8% and 34.3% in *P. aeruginosa* (Table 2). Susceptibility of *P. aeruginosa* to colistin was 92.5%. Intermediate susceptibility against piperacillin, piperacillin/tazobactam, cefepime, ceftazidime, imipenem, meropenem and aztreonam in *P. aeruginosa* isolates were 10.2%, 5.1%, 6.1%, 4.1%, 3.2%, 8.3% and 42.2% respectively. Intermediate susceptibility against tigecycline in *A. baumannii* was observed as 16.6%. The antibiotic resistance rates of non-fermenter Gram-negative rods were given in Table 2. As seen in this table, 6.5% of *S.maltophilia* and 4.4% of *B.cepacia* isolates were resistant to trimethoprim/sulfamethoxazole which is the first choice of use against such isolates.

Enterobacterales members accounted for 23.9% (7189/30,051) of the total LRT bacterial isolates. The most common *Enterobacterales* species were *K. pneumoniae* (N = 5140/7189) and *E. coli* (N = 2377/7189) respectively. Imipenem and meropenem resistance rates were 53.1% and 48.1% for *K. pneumoniae* while 4.3% and 5.1% for *E. coli* 12.0% and 10.1% for *Enterobacter* spp. Colistin was the most

Table 2. Resistance Rates of Non-Fermenter Gram-negative Rods.

	% R				
	A.baumannii	*P.aeruginosa*	*OtherNF**	*S.maltophilia*	*B.cepacia*
Piperacillin	96.2	37.7	35.7		
PTZ	91.6	31.7	30.6		
Cefepime	95.1	29.5	44.1		
Ceftazidime	94.2	31.5	26.7	79.7	29.9
Imipenem	92.8	39.8	25.8		
Meropenem	93.1	34.3	24.9		20.3
Ciprofloxacin	93.4	32.8	26.7		
Levofloxacin	91	36.4	20.6	7.1	7
Amikacin	77.6	19.9	34.3		
Gentamicin	78.9	29.6	35.6		
Tobramycin	60.8	14.7	32.8		
Netilmicin	75.7	33.2	34.3		
STX	75.6		23.2	6.5	4.4
Colistin	12.8	7.5			
Tigecycline	18.6	85.2	16		

*NF: Non-fermenter; TZP: piperacillin/tazobactam; SXT: trimethoprim/sulfamethoxazole.

Table 3. Resistance rates of *Enterobacterales* members.

	E. coli	K. pneumoniae	Enterobacter spp.	Serratia spp.	Proteus spp.	Klebsiella spp.	Citrobacter spp.	Other
					R%			
Amikacin	4.4	32.6	6.2	3.1	9.0	1.8	0	19.5
Gentamicin	32.7	50.6	10.9	6.8	42.5	5.8	5.6	37.9
Netilmicin	29.5	41.6	16.6	22.5	29.9	23.1	12.5	33.3
Cefoxitin	21.9	44.6	-	-	15.5	47.9	-	26.9
Cefuroxime	66.8	76.6	69.3	-	29.6	32.7	40.0	45.1
Cefotaxime	55.7	80.8	41.3	52.8	16.7	-	50.0	0
Ceftriaxone	62.0	72.6	30.9	27.4	39.9	24.8	23.0	34.9
Ceftazidime	57.9	71.1	29.0	17.0	16.8	19.9	19.4	27.1
Cefepime	51.6	66.7	17.3	9.6	25.4	8.3	11	23.3
Levofloxacin	63.6	52.9	12.6	18.6	40.0	33.3	9.1	-
Ciprofloxacin	59.7	60.6	12.2	9.4	49.1	10.8	11.7	44.2
Ertapenem	7.5	51.1	13.8	10.6	9.0	7.9	4.7	20.7
Imipenem	2.9	47.3	8.3	7.4	9.0	14.5	8.6	12.1
Meropenem	3.5	45.1	7.6	6.1	4.8	4.6	2.7	14
Colistin	1.7	18.5	5.8	-	-	1.1	-	-
SXT*	54.8	55.9	12.8	4.2	69.4	11.3	15.1	41.4
Tigecycline	2.4	19.8	4.6	-	16.7	84.6	20.0	3.0

*SXT: trimethoprim/sulfamethoxazole.

effective antibiotic against *K. pneumoniae* and *E. coli* (Table 3). Resistance rates of colistin differ from center to center between 0%-4.4% (1.4% SD) for *E. coli* strains and 4.4%-28.5% (7.3% SD) for *K. pneumonia.* ESBL rate was detected as 62.5% for *K. pneumoniae;* 53.1% for *E. coli*; 19.1% for other *Klebsiella* species; 13.9% for *Enterobacter* spp.; 8.6% for *Proteus* spp.; 6.3% for *Citrobacter* spp.; and 4.3% for *Serratia* spp.

Among staphylococci, 27.3% of *S. aureus* and 82.4% of coagualse-negative staphylococci (CoNS) were methicillin resistant ($p < 0.0001$). Among

enterococci, 7.6% of *E. faecium* and 0.9% of *E. faecalis* were resistant to vancomycin ($p = 0.0024$). Linezolid resistant *S. aureus*, CoNS, *E. faecalis* and *E. faecium* rates were 0.3%, 2.9%, 0.0% and 4.6% respectively (Table 4). Inducible clindamycin resistance rates were 17.2% in *S. aureus* and 38.2% in CoNS ($p < 0.0001$). Penicillin non-susceptible *S. pneumoniae* rate was 37.0%. Resistance rates of Gram-positive isolates and intermediate resistance rates for *S. pneumoniae* against certain antibiotics were given at Table 4.

Table 4. Resistance rates of Gram-positive isolates.

	S. aureus	CoNS	p	E. faecalis	E. faecium	p	%R	%I
			R %				S. pneumoniae	
Penicillin	81.3	98.3	< 0.001				25.9	11.1
Ampicillin				16.3		< 0.001	23.5	7.0
Levofloxacin	16.7	68.8	< 0.001	59.3	100	0.0452	9.7	0.2
Ciprofloxacin	18.8	74.4	< 0.001	51.5	90.2	< 0.001		
Moxifloxacin	26.9	83.3	0.0351				10.2	0.2
Vancomisin	0.0	0.0		0,97	7.6	0.0024		
Gentamicin	15.2	64.6	< 0.001					
Erythromycin	25.4	80.5	< 0.001				45.2	0.8
Klindamycin	23.1	69.1	< 0.001				34.5	0.5
Linezolid	0.3	2.9	< 0.001	0.0	4.6	0.0055		
Tetracycline	26.2	68	< 0.001					
SXT*	4.5	33.1	< 0.001	40.0	40.2	0.9233	30.1	2.0
HL**-Gentamicin				36.9	48	0.1415		
Methycillin	27.2	81.9	< 0.001					
Cefotaxime							16.4	9.2
Imipenem							4.5	1.1
Meropenem							8.1	3.2
Chloramphenicole							5.9	
Rifampicin							11.4	

* SXT: trimethoprim/sulfamethoxazole; ** HL: high Level.

Two common CA isolates of LRTIs were *H. influenzae* and *M. catarrhalis.* Their antibiotic resistance rates were given in Table 5.

Discussion

The rise in antimicrobial resistance is a growing public health concern impacting on morbidity, mortality and costs, calling for urgent action as local problems with resistance become a global threat [14-16]. As resistance mechanisms and the dissemination of resistance vary widely, it is important to provide accurate and local information on the prevalence of antimicrobial resistance among the causative pathogens of respiratory tract infections [2]. Some information with limited number of isolates is available on antimicrobial resistance from many countries. There are not many extensive studies dealing with resistance rates of respiratory pathogens. We believe that documenting etiology of LRTIs and resistance profile is very important as these strains may cause outbreaks particularly in ICUs, limit therapeutic options, and lead increased morbidity, mortality and financial burden. This retrospective multicenter analysis consists of the data generated from routine diagnostic laboratory of 17 participant hospital including city hospital, training and research hospital and university hospitals from 11 cities around the country. To our knowledge, this is the first and the largest multicenter report from Turkey on bacterial etiology of LRTIs and their resistance profile.

Gram-negatives (85.1%) were seen more than Gram-positives (14.9%) as causative pathogen of LRTIs ($p < 0.0001$). The role of Gram-negative in the etiology of LRTI has increased probably because of rapid colonization of hospitalized patients with Gram-negative bacilli. *A. baumannii*, an emerging multidrug-resistant (MDR) pathogen, was the most prevalent isolate (25.1%), responsible for both CA and HA infections. The rate of *A. baumannii* in LRTIs was significantly higher in HA infections than CA ($p < 0.0001$). *A. baumannii* in current study exhibited significantly more resistance profile comparing with *P. aeruginosa* and other non-fermenter Gram-negative bacilli ($p < 0.0001$). The resistance rates to imipenem and meropenem were detected as 92.8% and 93.1% in *A. baumannii* isolates respectively. Since the first report of imipenem resistance in 1987, prevalence of resistance rate to carbapenems in *A. baumannii* in Europe countries has reached 85% in Greece, 50-80% in Turkey, 60% in Italy in 2007 [17], and currently it is reported as 95% in Serbia, 91% in WHO 2018 Central Asian and Eastern European Surveillance report [12]. Similar rates against carbapenems were also observed in *A. baumanni* isolates from LRTIs in this study. Not only to carbapenems, *A. baumannii* also showed high resistance rates to cephalosporins and quinolones (Table 2). Although 3rd cephalosporin+macrolide or 3rd cephalosporin+levofloxacin combinations are recommended for severe group [18] high level of resistance for these antibiotics is worrying. Highest total cephalosporin usage and highest use of third-generation quinolones in Turkey among Eastern Europe countries [19] might have led the high level of resistance against these antibiotics. Colistin is the last resort for treatment of MDR *A. baumannii* and

Table 5. Resistance rates of *H. influenzae* and *M. catarrhalis* isolates.

	%R	
	H.influenzae	*M.catarrhalis*
Penicillin	53.6	
Ampicillin	48.6	71.8
SAM	26.5	63.6
AMC	35.7	38.5
Cefepime	7.9	6.0
Cefuroxime	27.9	9.2
Cefotaxime	9.9	3.1
Cefixime	8.5	7.8
Levofloxacin	11.7	1.1
Ciprofloxacin	14.3	3.4
Moxifloxacin	17.6	2.0
Ofloksasin	17.9	0.0
Erythromycin	25.7	5.9
Tetracycline	18.4	4.2
Chloramphenicole	2.8	2.6
Rifampicin	45.2	36.0
STX	37.3	22.1

AMC: Ampicillin/Sulbactam; AMC: Amoxicillin/Clavulonic acid; SXT: trimethoprim/sulfamethoxazole.

unfortunately, resistance to colistin has been reported all over the world. The highest resistance rate was reported in Asia, followed by Europe. Reports from Bulgaria and Spain showed high rates as 16.7% and 19.1%, respectively. Asia and Europe already showed the most serious situation of colistin resistance [20] and resistance rate to colistin was detected as 12.8% in our study.

In total, 21.7% of all isolates were *P. aeruginosa*. Most HA *P. aeruginosa* were isolated from TA (68.3%) of and most CA *P. aeruginosa* were isolated from sputum (67.4%). *P. aeruginosa,* as a causative pathogen, has similar impact on HA and CA LRTIs (p=0.0703). MYSTIC surveillance study reported carbapenems, piperacillin-tazobactam and tobramycin as the most effective antibiotics against *Pseudomonas* isolates [21], in this study these rates were found as 34.3%, 31.7% and 14.7% respectively, and the lowest resistance rate was observed against colistin (7.1%). Carbapenems were not detected as the most effective antibiotics against *P. aeruginosa* isolates in this study.

K. pneumoniae, a member of *Enterobacteriaceae* family, is another pathogen that a common cause of LRTIs. Like *A. baumannii, K. pneumoniae* was also isolated more frequently as HA pathogens (p<0.0001). Comparing with other *Enterobacterales* members they showed significantly higher resistance pattern (p<0.0001). The most effective antibiotic was colistin, and 18.9% of *K. pneumoniae* were resistant against colistin, higher than *A. baumannii*. Carbapenem non-susceptible *K. pneumoniae* rate was reported as 38% in CAESAR surveillance report of blood and CSF isolates [12] and this current study showed that 47.3% and 45.1% of *K. pneumoniae* isolates were resistant to imipenem and meropenem which means respiratory tract isolates of *K. pneumoniae* had higher resistance rate than blood and CSF isolates.

In *Enterobacteriaceae,* ESBLs and/or hyperproduction of AmpC beta-lactamases are cause of resistance to third- and fourth-generation cephalosporins beside [22]. *K. pneumoniae* was the most ESBL producers (63.1%) followed by *E. coli* (53%) and statistically more than the other members of *Enterobacterales* (p<0.0001). The inappropriate empirical use of broad-spectrum beta-lactams particularly third- and fourth-generation cephalosporins, carbapenems, penicillin, and beta-lactamase inhibitor combinations, or quinolones either alone or in combination with an aminoglycoside and/or a glycopeptide might have increased the resistance rates of ceftazidime, cefepim, carbapenems, quinolones as seen in table 2 and 3. AmpC beta-lactamases in *K.*

pneumoniae and *E. coli* raise concerns over the spread of resistance [23]. Cefoxitin was assessed as primary screening marker of plasmid encoded AmpC might be the phenotypic implication of this mechanism. Cefoxitin resistance rate was 21.9% in *E. coli* and 44.6% in *K. pneumoniae* isolates. In this study; centers did not use confirmatory AmpC test but the sensitivity and specificity of cefoxitin for the detection of AmpC productions 97.4% and 78.7% respectively [24]. For both ESBL- and AmpC-producing isolates, the carbapenems meropenem and imipenem remain first-line agents for the treatment of such infections since they are the only agents active against both resistance mechanisms [23]. But increased resistance rates in carbapenem group of antibiotics are worrying.

An environmental Gram-negative non-fermenter *S. maltophilia* is the most commonly associated bacteria with respiratory infections [25]. The rate of *S. maltophilia* in LRTIs was found as 1.26% (N = 380) among all isolates and 6.7% of them were resistant to SXT. Results from the SENTRY Antimicrobial Surveillance Program in 2004 showed that the resistant rate to TMP-SMX was 3.8% in *S. maltophilia* [8], and results from surveillance program showed a level of resistance up to 10% across Europe and a study showed that 5.87% of *S. maltophilia* isolates were reported as extensively drug resistant [26]. The preferred treatment of *S. maltophilia* infections is TMP-SXT and our study showed similar SXT resistance rate with less than 10%.

Burkholderia cepacia can cause opportunistic and hospital acquired LRTIs, usually among cystic fibrosis (CF) patients and responsible for approximately 0.6% of all ventilator-associated pneumonias [27]. *B. cepacia* infections emerged as a problem among persons with CF and other immunocompromised individuals [28] and it is resistant to many antimicrobial agents because of the innate and acquired mechanisms of resistance. Resistance rate of *B. cepacia* to the commonly used antibiotics; ceftazidime, meropenem, and trimethoprim-sulfamethoxazole were found as 29.9%, 20.3% and 4.4% respectively.

Community-acquired respiratory tract infections have been complicated by the emergence in three major pathogens: *S. pneumoniae* (19.9%), *H. influenzae* (14.6) and *M. catarrhalis* (3.5%). They were more frequently isolated as causative pathogen of community-acquired LRTIs in current study (Table 1). *S. pneumoniae* continues to be responsible for respiratory tract infections and it was known that around 20-30% of all pneumonias are caused by multidrug-resistant strains of *S. pneumoniae*, and about the 30-40% are penicillin-resistant [29]. CAP is most

commonly caused by *S. pneumoniae*, and resistance to penicillin is an emerged problem. The rate of penicillin resistant *S. pneumoniae* in a surveillance study was reported among isolates from Asia (18.9%) [30]. Susceptibility testing results from eight European countries showed that penicillin G non-susceptible *S. pneumoniae* rate was 24.6% and macrolide resistant rate was 28.0%. Fluoroquinolones are also recommended for initial empirical therapy of selected outpatients with community-acquired respiratory tract infections [31]. In this study, *S. pneumoniae*, resistance rates were as follows: penicillin intermediate, 11.1%; penicillin resistant, 25.9%; erythromycin, 45.2%; and levofloxacin, 9.7% (Table 4). Resistance to β-lactams was seen most common with macrolide resistance. The different trends in resistance development over time should be considered to offer alternatives to β-lactams for the treatment of lower RTIs caused by *S. pneumoniae.*

H. influenzae is an important pathogen causing respiratory tract infections and invasive diseases. Ampicillin remains the first-line drug of choice for *H. influenzae* infections Ampicillin resistance in our study was found as 48.6%. In a European study, more than 10% of the *H. influenzae* strains were β-lactamase producers. Additionally, there can be enormous regional differences and changes in β-lactamase prevalence, ranging from 3% in Germany up to 65% in Korea [6]. A limitation of present study was β-lactamase test results were not available in all participant centers. Because of high β-lactamase rates in *H. influenzae*, amoxicillin-clavulanate and oral cephalosporins have been widely used for oral antibiotic treatments for outpatients [6]. The resistance rate to amoxicillin-clavulanate was detected as 35.7% in our study. In the present study, erythromycin resistance rate was 25.7% and levofloxacin resistance rate was 11.7%. Assessing Worldwide Antimicrobial Resistance Evaluation program reported that susceptibility to levofloxacin among β-lactamase-negative and -positive *H. influenzae* (87.2% and 77.3%, respectively) was lower in Africa and Middle East compared with other regions [30].

Moraxella catarrhalis is an important pathogen that is a major cause of variety of respiratory tract infections [32]. Almost 90% of clinical isolates of *M. catarrhalis* has been producing β-lactamase. Ampicillin and amoxicillin-clavulanate resistance rates were 71.8% and 38.5%, respectively in the present study. Other enzyme-stable β-lactams, macrolides, and tetracyclines are still active against *M. catarrhalis*, however it was reported that rates of SXT resistance has reached 50%

rate. Additionally, *M. catarrhalis* isolates resistant to several fluoroquinolones, including levofloxacin, have been described [33]. The resistance rate to erythromycin was 5.9% and the resistance rates to fluoroquinolones were about 1-3% in our results.

It was known the apparent colonization ability of the respiratory tract by enterococci and they rarely cause respiratory tract infections. However, they have emerged as an increasingly important cause of hospital acquired infections currently [34]. In this study, 349 enterococci were isolated as causative pathogen of LRTIs and 344 (98.6%) were hospital acquired isolate. *Enterococcus faecalis* and *E. faecium* responsible for the majority of human infections and we did not detect any other enterococci species in this study. WHO has listed vancomycin-resistant (VRE) *E. faecium* as a pathogen with high priority in its global priority list of antibiotic-resistant bacteria [12]. Vancomycin resistance in *E. faecium* (7.6%) was significantly higher than in *E. faecalis* (0.96%) (*p* = 0.0024. Ampicillin resistance in E. *faecium* (97.1%) was also observed higher comparing with *E. faecalis* (*p* < 0.0001). An increase in the number of infections caused by ampicillin-resistant *E. faecium* has also been observed in many countries. The clonal complex characterized by ampicillin resistance has been associated with nosocomial outbreaks in five continents [35]. HLGR was observed 36.9% and 48.0% in *E. faecalis* and *E. faecium* respectively (*p* = 0.1415). HLGR rate was 30% (29-31) in European countries as reported in EARS-NET [36].

Conclusions

Bacteria that are causing LRTIs are increasingly become resistant to previously effective antibiotics. While effective medical and public health practice will hopefully prevent the arrival of a "post-antibiotic" era the continuously diminishing number of drugs effective against *A. baumannii*, *K. pneumoniae*, *P. aeruginosa S. pneumoniae,* and other common pathogens of CA and HA pneumonias raise concern. Our results have once again demonstrated the necessity of implementing rational antibiotic use policies by antibiotic control committees in order to avoid increasing resistance rates and to prevent resistant bacteria spread.

Gurbuz, Aysel Karatas, Selcuk Kaya, Canan Kulah, Hasan Cenk Mirza, Melda Ozdamar, Birol Safak, Pınar Sagiroglu, Ali Korhan Sig, Berksan Simsek.

References

1. Mizgerd JP (2006) Lung Infection - A Public Health Priority. PLoS Med 3: e76.
2. Jacobs E, Dalhoff A, Korfmann G (2009) Susceptibility patterns of bacterial isolates from hospitalised patients with respiratory tract infections (MOXIAKTIV Study). International Journal of Antimicrobial Agents 33: 52–57.
3. Mizgerd JP (2008) Acute Lower Respiratory Tract Infection. N Engl J Med 358: 716–727.
4. Aslam A, Gajdács M, Zin CS, Binti Abd Rahman NS, Ahmed SI, Jamshed SQ (2020) Public Awareness and Practices towards Self-Medication with Antibiotics among the Malaysian Population. A Development of Questionnaire and Pilot-Testing. Antibiotics 9: 97.
5. Felmingham D, Gruneberg RN, The Alexander Project Group (1996) A multicentre collaborative study of the antimicrobial susceptibility of community-acquired, lower respiratory tract pathogens 1992-1993: The Alexander Project. Journal of Antimicrobial Chemotherapy 38: 1–57.
6. Bae S, Lee J, Lee J, Kim E, Lee S, Yu J, Kang Y (2010) Antimicrobial Resistance in Haemophilus influenzae Respiratory Tract Isolates in Korea: Results of a Nationwide Acute Respiratory Infections Surveillance. Antimicrobial Agents and Chemotherapy 54: 65–71.
7. Gajdács M, Paulik E, Szabó A (2020) Knowledge, attitude and practice of community pharmacists regarding antibiotic use and infectious diseases: A Cross-Sectional Survey in Hungary (KAPPhA-HU). Antibiotics 9: 41.
8. World Health Organization, Global Action Plan on Antimicrobial Resistance. Available: http://www.who.int/antimicrobial-resistance/global-action-plan/en/. Accessed 14 October 2019.
9. Farrell DJ, Morrissey I, Bakker S, Felmingham D (2002) Molecular characterization of macrolide resistance mechanisms among Streptococcus pneumoniae and Streptococcus pyogenes isolated from the PROTEKT 1999-2000 study. Journal of Antimicrobial Chemotherapy 50: 39–47.
10. Fedler KA, Biedenbach DJ, Jones RN (2006) Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: Report from the 2004 SENTRY Antimicrobial Surveillance Program on 3 continents. Diagnostic Microbiology and Infectious Disease 56: 427–436.
11. Gajdács M, Urbán E (2019) Epidemiological trends and resistance associated with Stenotrophomonas maltophilia bacteremia: a 10-year retrospective cohort study in a tertiary-care hospital in Hungary. Diseases, 7: 41.
12. World Health Organization, Central Asian and Eastern European Surveillance of Antimicrobial Resistance, Annual report 2017 (2018) Available: http://www.euro.who.int/en/health-topics/disease-prevention/antimicrobial-resistance/publications/2017/central-asian-and-eastern-european-surveillance-of-antimicrobial-resistance.-annual-report-2017-2018. Accessed 14 October 2019.
13. Esen B, Albayrak N, Aliskan HE, Arikan S, Cicek C, Erdem G, Esen N, Korkmaz M, Otgun SN, Yucel M (2015) Application Guide from Clinical Sample to Final Report;

Respiratory System Examples. Clinical Microbiology Specialist Association. 10: 54–60. Available: https://www.klimud.org/public/uploads/files/solunum-sistemi-ornekleri.pdf. Accessed 01 September 2019.
14. Gandra S, Barter DM, Laxminarayan R (2014) Economic burden of antibiotic resistance: how much do we really know? Clin Microbiol and Infect 20: 973–980.
15. Michael CA, Dominey-Howes D, Labbate M (2014) The Antimicrobial Resistance Crisis: Causes, Consequences, and Management. Front Public Health 2: 145.
16. Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, Knight GM, Robotham JV (2018) Estimating the burden of antimicrobial resistance: a systematic literature review. Antimicrob Resist Infect Control 25;7: 58.
17. Kempf M, Rolain J-M (2012) Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents 39: 105–114.
18. Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Leven M, Ortqvist A, Schaberg T, Torres A, van der Heijden G, Read R, Verheij TJM (2011) Guidelines for the management of adult lower respiratory tract infections - Full version. Clin Microbiol Infect 17: E1–E59.
19. Versporten A, Bolokhovets G, Ghazaryan L, Abilova V, Pyshnik G, Spasojevic T, Korinteli I, Raka L, Kambaralieva B, Cizmovic L, Carp A, Radonjic V, Maqsudova N, Celik HD, Payerl-Pal M, Pedersen HB, Sautenkova N, Goossens H (2014) Antibiotic use in Eastern Europe: a cross-national database study in coordination with the WHO Regional Office for Europe. Lancet Infect Dis 14: 381–387.
20. Cai Y, Chai D, Wang R, Liang B, Bai N (2012) Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 67: 1607–1615
21. Rhomberg PR, Jones RN (2007) Contemporary activity of meropenem and comparator broad-spectrum agents: MYSTIC program report from the United States component (2005). Diagn Microbiol Infect Dis 57: 207–215.
22. Denys GA, Relich RF (2014) Antibiotic Resistance in Nosocomial Respiratory Infections. Clin Lab Med 34: 257–270.
23. Pfaller MA, Jones RN, MYSTIC Study Group (Europe) (2002) Antimicrobial susceptibility of inducible AmpC beta-lactamase-producing Enterobacteriaceae from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme, Europe 1997-2000. Int J Antimicrob Agents 19: 383–388.
24. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Böttger EC, Hombach M (2011) Practical approach for reliable detection of AmpC beta-lactamase-producing Enterobacteriaceae. J Clin Microbiol 49: 2798–2803.
25. Brooke JS (2012) Stenotrophomonas maltophilia: an Emerging Global Opportunistic Pathogen. Clinical Microbiology Reviews 25: 2–41.
26. Gajdács M. Urbán E (2019) Prevalence and antibiotic resistance of Stenotrophomonas maltophilia in respiratory tract samples: a 10-year epidemiological snapshot. Health Serv Res Manag Epidemiol 6: 2333392819870774.
27. Ramsey AH, Skonieczny P, Coolidge DT, Kurzynski TA, Proctor ME, Davis JP (2001) *Burkholderia cepacia* Lower Respiratory Tract Infection Associated With Exposure to a Respiratory Therapist. Infect Control Hosp Epidemiol 22: 423–426.

28. Govan JRW, Hughes JE, Vandamme P (1996) Burkholderia cepacia: medical, taxonomic and ecological issues. J Med Microbiol 45: 395–407.

29. Martinez EP, Cepeda M, Jovanoska M, Bramer WM, Schoufour J, Glisic M, Verbon A, Franco OH, Yahav D (2019) Seasonality of antimicrobial resistance rates in respiratory bacteria: A systematic review and meta-analysis. PLoS ONE 14: e0221133.

30. Bae I-G, Stone GG (2019) Activity of ceftaroline against pathogens associated with community-acquired pneumonia collected as part of the AWARE surveillance program, 2015–2016. Diagn Microbiol Infect Dis 95: 114843.

31. Reinert RR, Reinert S, van der Linden M, Cil MY, Al-Lahham A, Apperbaum P (2005) Antimicrobial Susceptibility of Streptococcus pneumoniae in Eight European Countries from 2001 to 2003. Antimicrob Agents Chemother 49: 2903–2913.

32. Verduin CM, Hol C, Fleer A, van Dijk H, van Belkum A (2002) Moraxella catarrhalis: from emerging to established pathogen. Clin Microbiol Rev 15: 125–144.

33. Hoban DJ, Doern GV, Fluit AC, Roussel-Delvallez M, Jones RN (2001) Worldwide Prevalence of Antimicrobial Resistance in Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis in the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis 32: S81-S93.

34. Sood S, Malhotra M, Das BK, Kapil A (2008) Enterococcal infections & antimicrobial resistance. Indian J Med Res 128: 111-121.

35. Leavis HL, Bonten MJM, Willems RJL (2006) Identification of high-risk enterococcal clonal complexes: global dispersion and antibiotic resistance. Curr Opin Microbiol 9: 454–460.

36. European Centre for Disease Prevention and Control, Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Available: https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2017 Accessed 11 November 2019.

Corresponding author
Professor Ahmet Celal Basustaoglu, MD.
Baskent University, Faculty of Medicine
Head of the Department of Medical Microbiology,
Baglica Campus, Ankara, TURKEY
Tel: 90 532 471 29 92
Fax: 90 312 246 66 89
Email: basustaoglu@gmail.com

Conflict of interests: No conflict of interests is declared.