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Original Article

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Prevalence and Antimicrobial Resistance Trends among Lower Respiratory Tract Pathogens in Crete, Greece, 2017-2022

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ABSTRACT

Background: Lower respiratory tract infections (LRTIs) are the most common infections in humans accounting for significant morbidity and mortality. Management of LRTIs is complicated due to increasing antimicrobial resistance. This study investigated the prevalence and trends of antimicrobial resistance for bacteria isolated from respiratory samples of patients with LRTIs.

Materials and Methods: Sputum and bronchial washings were collected from patients of all ages hospitalized with LRTIs and were analyzed by the microbiological laboratory in the University Hospital of Heraklion, Crete, Greece, from January 2017 to December 2022. Identification of the bacterial isolates was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry and antimicrobial susceptibility testing by Vitek 2 system.

Results: A total of 4,008 strains were isolated from 3,427 respiratory samples. *Acinetobacter baumannii* was the most frequently isolated pathogen (23.1%), followed by *Pseudomonas aeruginosa* (20.0%), *Staphylococcus aureus* (10.6%) and *Klebsiella pneumoniae* (6.8%). The isolation rate of *A. baumannii* significantly increased during the study period, while there were lower increases in the isolation rates of *P. aeruginosa*, *K. pneumoniae* and *S. aureus*. *A. baumannii* and *P. aeruginosa* were more prevalent during summer, *K. pneumoniae* was more common during autumn, while for *S. aureus* higher incidence was noted during winter. *A. baumannii* exhibited high resistance rates (≥90.0%) to most of the antimicrobial agents tested, and extremely high multidrug-resistance (91.0%). *P. aeruginosa* showed the lowest rate of resistance for colistin (1.4%). Among β-lactams, resistance rates to piperacillin/tazobactam, ceftazidime, cefepime, imipenem and meropenem were 26.2%, 27%, 25.8%, 29.2% and 29.9%, respectively. A total of 162 (68.1%) meropenem-resistant *P. aeruginosa* were simultaneously resistant to ceftazidime and piperacillin/tazobactam. Regarding *K. pneumoniae*, high rates of resistance were observed for the third and fourth generation cephalosporins, namely cefotaxime, ceftriaxone, ceftazidime, and cefepime and the carbapenems, imipenem and meropenem ranging from 46.2% to 53.8%. Carbapenem-resistance was detected among 46.2% of the isolates. Among the 126 carbapenem-resistant *K. pneumoniae* isolates, 83 (65.9%), 30 (23.8%), 9 (7.2%), and 4 (4.2%) were

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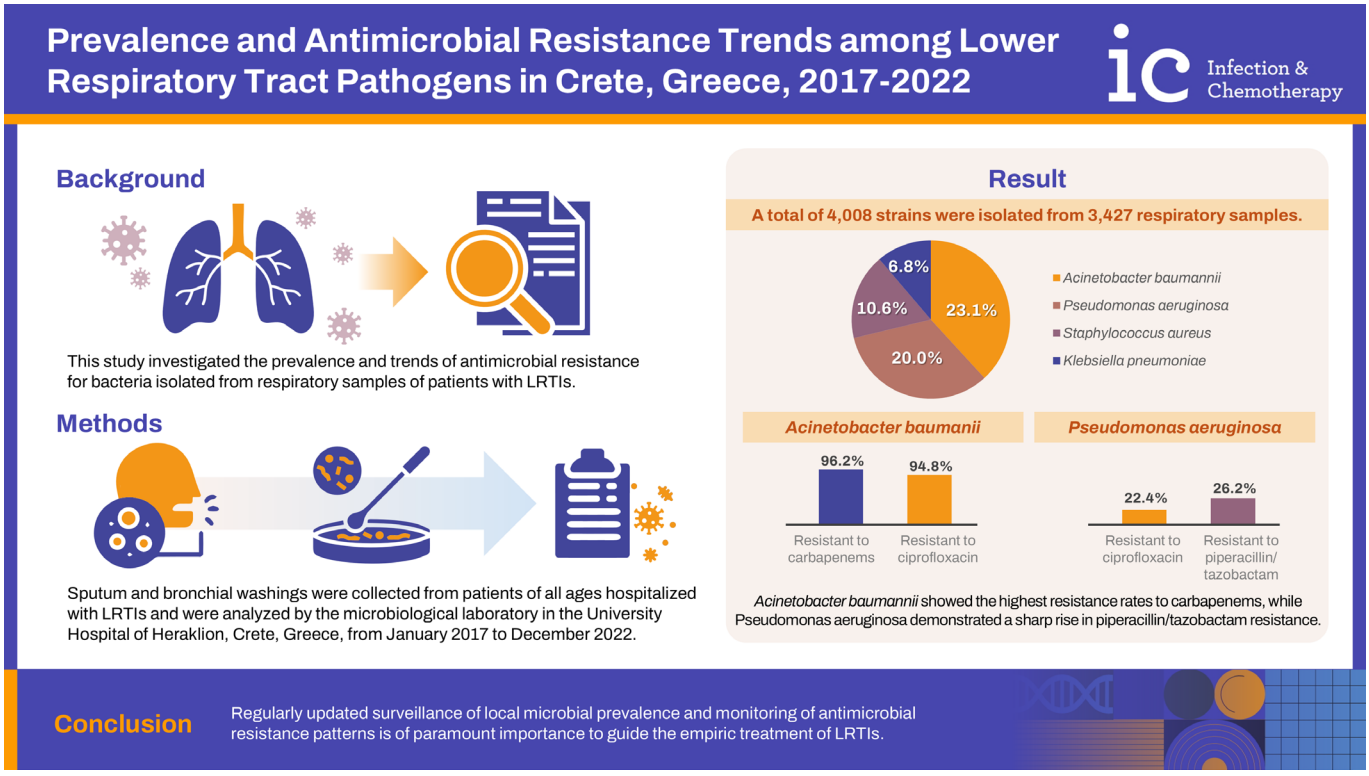
positive for *Klebsiella pneumoniae* carbapenemase, New Delhi Metallo-β-lactamase, Verona Integron-Mediated Metallo-β-lactamase and OXA-48 carbapenemase, respectively. Of the total number of *S. aureus*, 37.2% were methicillin resistant. Low rates of resistance were detected in trimethoprim/sulfamethoxazole (3.3%), gentamicin (2.8%), and rifampicin (0.9%). All isolates were susceptible to linezolid, daptomycin, tigecycline, teicoplanin, and vancomycin. **Conclusion:** Regularly updated surveillance of local microbial prevalence and monitoring of antimicrobial resistance patterns is of paramount importance to guide the empiric treatment of LRTIs.

Keywords: Lower respiratory tract infections; *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*; *Staphylococcus aureus*

GRAPHICAL ABSTRACT



INTRODUCTION

Lower respiratory tract infections (LRTIs) represent one of the most common infectious diseases worldwide [1]. Globally, in 2019 there were 488.9 million incident cases and 2.4 million deaths due to LRTIs [2]. According to the Global Burden of Disease 2021 study, LRTIs represent the seventh most common causes of death in the world [3]. Mortality from LRTIs in Greece increased from 5.09 deaths/100,000 population for 1998 to 10.20 deaths/100,000 population for 2015 [4]. LRTIs include acute bronchitis and bronchiolitis, pneumonia, and acute exacerbation of chronic lung diseases such as chronic obstructive pulmonary disease or bronchiectasis. Pneumonia, one of the most important LRTIs, is reported to affect approximately 450 million people a year [5]. Bacteria are the dominant pathogens of LRTIs, followed by other causative agents such as viruses, mycoplasma, chlamydiae, rickettsiae, and fungi. The most frequently isolated microorganisms from respiratory samples of patients with LRTIs include Gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Haemophilus influenzae* and Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae*.

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Empirical treatment plays an important role in the management of LRTIs. Therefore, knowledge of the potential causative agents and their antimicrobial susceptibility profile is very important. The increasing prevalence of antimicrobial resistance among these pathogens and the spread of multidrug-resistant (MDR) strains remains a global problem complicating the management of LRTIs. Treatment failure is associated with high morbidity, prolonged hospitalization, increased healthcare costs, and even increased mortality rates.

The frequency and antimicrobial susceptibility patterns of pathogens causing LRTIs vary over time and by geographic region and even from hospital to hospital. Empirical antimicrobial therapy rely on local patterns of antimicrobial resistance that should be continuously monitored in order to update treatment guidelines.

Greek data on antimicrobial resistance amongst pathogens recovered from LRTIs are limited [6, 7]. The present study investigated the prevalence of the most important bacterial pathogens responsible for LRTIs and their antimicrobial resistance trends over a 6-year period in Crete, Greece.

MATERIALS AND METHODS

1. Study setting and design

This is a retrospective single-center study of patients of all ages with LRTIs who were hospitalized from January 2017 to December 2022 in the University Hospital of Heraklion, Heraklion, Crete, Greece. The study hospital is a tertiary care hospital with 771 beds, serving a population of 700,000 people. Sputum and bronchial washings were collected and were promptly transported to the microbiological laboratory for further processing. One isolate per patient was identified and tested. Patients receiving antibiotics one week prior to sample collection and those with a respiratory specimen positive for acid-fast bacilli were excluded from the study. Patients' demographic and clinical characteristics included gender, and hospitalization ward.

The isolation frequency for each pathogen was calculated for each season. We defined March, April, and May as spring; June, July, and August as summer; September, October, and November as autumn, and December, January, and February as winter.

2. Ethics statement

This study was approved by the Ethical Committee of the University Hospital of Heraklion and met the guidelines of Helsinki declarations (protocol code 96352/10-05-2022).

3. Microbiology

1) Cultures and identification

Sputum and bronchoalveolar lavage fluid (BALF) were screened for initial quality regarding the presence of white blood cells and squamous epithelial cells, and subsequently were quantitatively cultured for aerobic microorganisms using chocolate agar, sheep blood agar, and MacConkey agar plates (BioMérieux, Marcy l'Etoile, France). The plates were incubated for 24–48 h at 36 °C. Colonies were then counted and bacterial concentrations (colony forming unit [CFU]/mL) were calculated. Diagnostic thresholds of $\geq 10^4$ CFU/mL and $\geq 10^7$ CFU/mL were adopted for BAL cultures and sputum, respectively. Bacterial identification was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (BioMérieux, France).

2) Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed by Vitek 2 system (BioMérieux), using the AST-N222 cards for *P. aeruginosa*, the AST- XN01 and AST-N233 cards for the other Gram-negatives and the AST-P659 for *S. aureus*. The antibiotics tested against the Gram-negatives included ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, cefotaxime, ceftriaxone, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, tetracycline, tigecycline, colistin, and trimethoprim-sulfamethoxazole (TMP/SMX). The antibiotics tested against *S. aureus* included penicillin, oxacillin, erythromycin, clindamycin, linezolid, daptomycin, teicoplanin, vancomycin, tetracycline, tigecycline, rifampicin, levofloxacin, and TMP/SMX.

Escherichia coli ATCC 25922, *K. pneumoniae* ATCC 700603, and *S. aureus* ATCC 29213 were used as quality control strains. The European Union Committee on Antimicrobial Susceptibility testing breakpoints were used to interpret colistin minimum inhibitory concentration (MIC) results, while the breakpoints proposed by the Clinical and Laboratory Standards Institute M100-Ed32 were used to interpret results for all other antimicrobials studied, except for tigecycline for which the U.S. Food and Drug Administration-recommended MIC breakpoints were applied [8, 9].

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MDR bacteria were defined as isolates non-susceptible to at least one agent in ≥3 antimicrobial categories and pandrug-resistant (PDR) bacteria were defined as isolates non-susceptible to all agents in all antimicrobial categories [10].

3) Phenotypic and molecular detection of carbapenemases

The immunochromatographic NG-Test Carba 5 (NG Biotech, Guipry, France) was used for the detection of the five most widespread carbapenemase families (*i.e.*, *Klebsiella pneumoniae* carbapenemase, New Delhi Metallo-β-lactamase, Verona Integron-Mediated Metallo-β-lactamase and OXA-48-like carbapenemase).

The presence of *bla* genes encoding for the presence of carbapenemases belonging to Ambler classes A, B, and D was confirmed by polymerase chain reaction with specific primers, as previously described [11].

4. Statistical analysis

Statistical analysis was conducted by χ^2 and Fisher's exact test, as appropriate. Statistical significance was set at P <0.05. All statistical analyses were performed with Graphpad Prism v.4 (GraphPad Software, San Diego, CA, USA).

RESULTS

1. Distribution and prevalence of bacterial pathogens

During the six-year period, a total of 4,008 strains were isolated from 3,427 samples of sputum (n=753) and BALF (n=2,674). The average incidence of BALF was 78% and that of sputum 22%. Of these pathogens, 3,458 (86.3%) were Gram-negative bacteria and 550 (13.7%) were Gram-positive bacteria. *A. baumannii*, *P. aeruginosa*, *Enterobacteriaceae*, and *S. aureus* accounted for 78.5% of the total isolates. *Enterobacteriaceae* were the most prevalent microorganisms (n=994; 24.8%) followed by *A. baumannii* (n=924; 23.1%), *P. aeruginosa* (n=801; 20.0%), and *S. aureus* (n=425; 10.6%). Among the 994 *Enterobacteriaceae* identified, *K. pneumoniae* was the most commonly isolated bacteria (27.5%). LRTIs bacterial pathogens are presented in **Table 1**. **Figure 1** shows the annual incidence of the most frequently isolated microorganisms from the samples of patients with LRTIs. The isolation rate of *A. baumannii* significantly increased during the study period (P=0.0001), while there were lower increases in the isolation rates of *P. aeruginosa*, *Enterobacteriaceae* and *S. aureus* (P=0.57, P=0.21, and

Table 1. Bacterial pathogens associated with lower respiratory tract infections

Isolates	No. (%)
Gram-negatives	3,458 (86.30)
Citrobacter braakii	3 (0.07)
C. freundii	10 (0.25)
C. koseri	25 (0.62)
Enterobacter aerogenes	45 (1.12)
E. cloacae	105 (2.61)
E. gergoviae	2 (0.05)
E. hormaechei	2 (0.05)
Escherichia coli	139 (3.46)
Hafnia alvei	5 (0.12)
Klebsiella aerogenes	37 (0.92)
K. oxytoca	38 (0.94)
K. pneumoniae	273 (6.80)
Morganella morganii	56 (1.40)
Proteus mirabilis	120 (3.00)
Providencia rettgeri	3 (0.07)
P. stuartii	4 (0.10)
Raoultella planticola	6 (0.15)
Serratia liquefaciens	7 (0.17)
S. marcescens	109 (2.71)
S. rubidaea	5 (0.12)
Achromobacter denitrificans	34 (0.84)
A. xylosoxidans	55 (1.37)
Acinetobacter baumannii	924 (23.10)
A. junii	3 (0.07)
A. lwoffii	6 (0.15)
A. pittii	5 (0.12)
A. ursingii	2 (0.05)
Aeromonas caviae	1 (0.02)
A. hydrophila	2 (0.05)
Alcaligenes faecalis	7 (0.17)
Alcaligenes xylosoxidans	4 (0.10)
Bordetella bronchiseptica	8 (0.20)
Burkholderia cepacia	7 (0.17)
Chryseobacterium gleum	3 (0.07)
Chryseomonas indologenes	12 (0.30)
Comamonas testosteroni	3 (0.07)
Delftia acidovorans	12 (0.30)
Elizabethkingia anophelis	2 (0.05)
E. meningoseptica	3 (0.07)
Haemophilus influenzae	194 (4.80)
Pasteurella multocida	4 (0.10)
Pseudomonas aeruginosa	801 (20.00)
P. fluorescens	24 (0.60)
P. luteola	26 (0.64)
P. putida	34 (0.84)
Sphingomonas paucimobilis	30 (0.74)
Stenotrophomonas maltophilia	241 (6.00)
Other Gram-negatives	17 (0.42)
Gram-positives	550 (13.70)
Corynebacterium striatum	15 (0.37)
Nocardia spp.	6 (0.15)
Rothia mucilaginosa	17 (0.42)
Staphylococcus aureus	425 (10.60)
Streptococcus pneumoniae	74 (1.84)
S. pyogenes	5 (0.12)
Other Gram-positives	8 (0.20)

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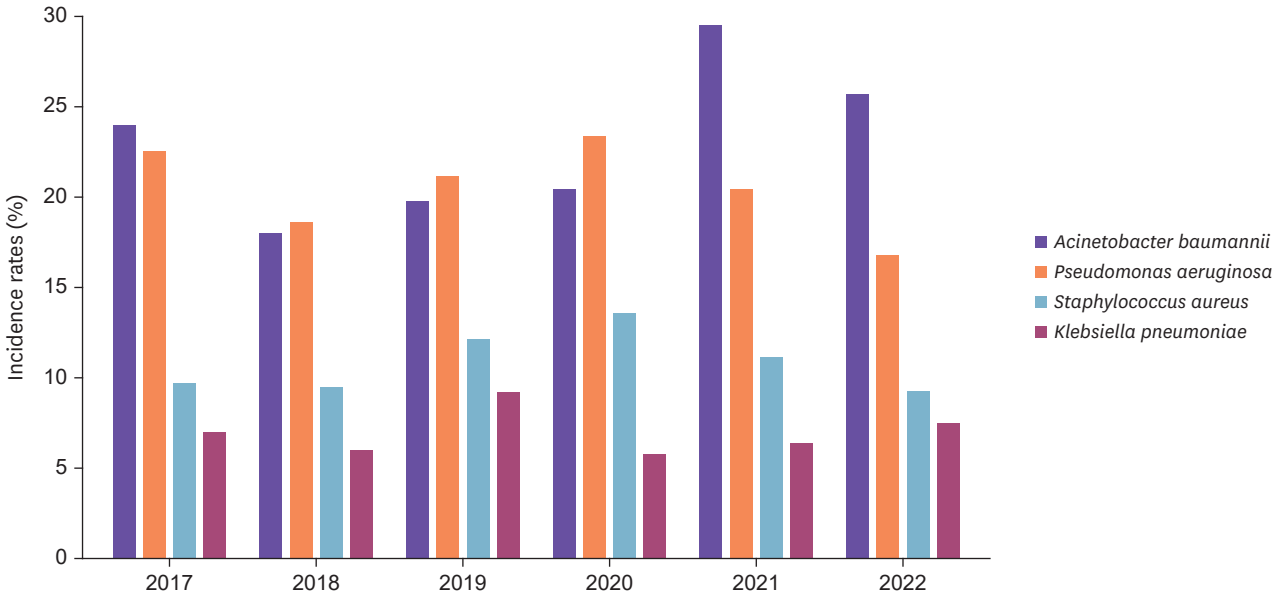

Figure 1. Annual incidence of the lower respiratory tract pathogens.

P=0.50, respectively). Isolation rates of *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* from samples of patients hospitalized in the Intensive Care Unit (ICU) were 54.2%, 36%, 47.3%, and 28.9%, respectively, while lower rates of 8.8%, 20.2%, 11.7%, and 25.2%, respectively were detected in patients of the Pulmonary Clinic. The number of isolates per positive sample presented in **Table 2**, shows that in 74.3% of cases there was monomicrobial growth, while in 25.7% there was polymicrobial growth. Higher rates of isolates were obtained from male patients (70.5%) compared to females (29.5%).

2. Seasonal distribution

A. baumannii and *P. aeruginosa* were more prevalent during summer, *K. pneumoniae* was more common during autumn, while for *S. aureus* higher incidence was noted during winter (**Fig. 2**).

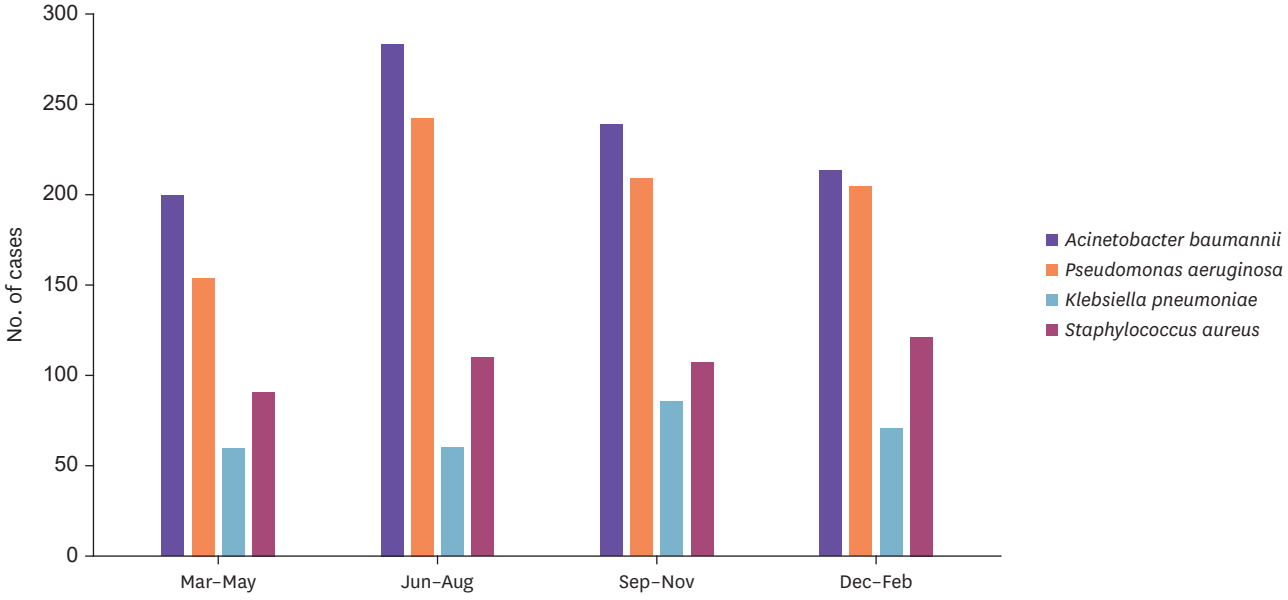

Figure 2. Seasonal distribution of the lower respiratory tract pathogens during 2017-2022.

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Table 2. Yearly percentage of the number of isolates per sample

Year	% of the number of isolates per sample		
	1	2	3
2017	61.2	33.7	5.1
2018	78.9	17.1	4.0
2019	86.5	11.4	2.1
2020	84.9	10.9	4.2
2021	74.6	19.6	5.8
2022	59.4	29.3	11.3

3. Antimicrobial susceptibility testing

Among the Gram-negative pathogens, *A. baumannii* exhibited extremely high resistance rates (≥90%) to most of the antimicrobial agents tested. Throughout the duration of the study, the lowest resistance rate was observed in tigecycline (53.8%) (**Table 3**). Resistance to carbapenems, aminoglycosides (amikacin, gentamicin, tobramycin) and TMP/SMX significantly increased from 93.8%, 85.8%, 90.0%, 90.0%, and 69.9% over the period 2017-2019 to 97.8%, 97.3%, 95.8%, 94.6%, and 89.1% over the years 2020-2022, respectively (*P*=0.002, *P* <0.001, *P*=0.001, *P*=0.009, and *P* <0.001, respectively) (**Table 3**). Eight hundred forty-one *A. baumannii* isolates (91%) were categorized as MDR and 35 (3.8%) as PDR.

P. aeruginosa was the second most frequently isolated pathogen from positive samples. During the six years (2017-2022) *P. aeruginosa* showed the lowest rate of resistance for colistin (1.4%). Among β-lactams, resistance rates to piperacillin/tazobactam, ceftazidime, cefepime, imipenem, and meropenem were 26.2%, 27%, 25.8%, 29.2%, and 29.9%, respectively. Resistance to piperacillin/tazobactam, ceftazidime, and cefepime increased from 23.3%, 23.6%, and 25.4% respectively, over the years 2017-2019, to 28.7%, 30.0%, and 26.2%, respectively, over the period 2020-2022 (*P*=0.014, *P*=0.051, and *P*=0.720, respectively) (**Table 4**). On the contrary, resistance to imipenem, meropenem, and the three aminoglycosides tested (amikacin, gentamicin, and tobramycin) decreased over the two three-year periods (*P*=0.854, *P*=0.383, *P*=0.468, *P*=0.316, and *P*=0.644, respectively) (**Table 4**). A total of 162 meropenem-resistant *P. aeruginosa* (68.1%) were simultaneously resistant to ceftazidime and

Table 3. Comparison of antimicrobial resistance rates in *Acinetobacter baumannii* isolates over the 2 study periods (2017-2019 and 2020-2022)

Antibiotic	2017-2019 (369 isolates)			2020-2022 (555 isolates)			*P*-value
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Ampicillin/sulbactam	10/96 (10.4)	11/96 (11.5)	75/96 (78.1)	2/81 (2.5)	6/81 (7.4)	73/81 (90.1)	0.060
Piperacillin/Tazobactam	8/138 (5.8)	-	130/138 (94.2)	6/130 (4.6)	-	124/130 (95.4)	0.786
Imipenem	23/369 (6.2)	-	346/369 (93.8)	12/542 (2.2)	-	530/542 (97.8)	0.002
Meropenem	23/369 (6.2)	-	346/369 (93.8)	12/542 (2.2)	-	530/542 (97.8)	0.002
Amikacin	43/302 (14.2)	-	259/302 (85.8)	15/550 (2.7)	-	535/550 (97.3)	<0.001
Gentamicin	30/369 (8.1)	7/369 (1.9)	332/369 (90.0)	20/550 (3.6)	3/550 (0.6)	527/550 (95.8)	0.001
Tobramycin	37/369 (10.0)	-	332/369 (90.0)	30/555 (5.4)	-	525/555 (94.6)	0.009
Colistin	60/369 (16.3)	-	309/369 (83.7)	103/555 (18.6)		452/555 (81.4)	0.379
Ciprofloxacin	23/369 (6.2)	-	346/369 (93.8)	8/230 (3.5)	-	222/230 (96.5)	0.184
Levofloxacin	27/369 (6.2)	3/369 (0.8)	339/369 (91.9)	8/230 (3.5)	2/230 (0.9)	220/230 (95.6)	0.149
Tigecycline	181/369 (49.1)		188/369 (50.9)	91/220 (41.4)	-	129/220 (58.6)	0.073
TMP/SXM	111/369 (30.1)	-	258/369 (69.9)	25/229 (10.9)	-	204/229 (89.1)	<0.001

TMP/SMX, trimethoprim-sulfamethoxazole.

Table 4. Comparison of antimicrobial resistance rates in *Pseudomonas aeruginosa* isolates over the 2 study periods (2017-2019 and 2020-2022)

Antibiotic	2017-2019 (373 isolates)			2020-2022 (428 isolates)			*P*-value
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Piperacillin/tazobactam	275/373 (73.8)	11/373 (2.9)	87/373 (23.3)	279/428 (65.2)	26/428 (6.1)	123/428 (28.7)	0.014
Ceftazidime	265/373 (71.0)	20/373 (5.4)	88/373 (23.6)	268/426 (62.9)	30/426 (7.1)	128/426 (30.0)	0.051
Cefepime	255/373 (68.4)	23/373 (6.2)	95/373 (25.4)	284/428 (66.4)	32/428 (7.4)	112/428 (26.2)	0.720
Imipenem	252/373 (67.6)	9/373 (2.4)	112/373 (30.0)	291/424 (68.7)	12/424 (2.8)	121/424 (28.5)	0.854
Meropenem	240/373 (64.3)	15/373 (4.0)	118/373 (31.7)	280/424 (66.0)	24/424 (5.7)	120/424 (28.3)	0.383
Amikacin	306/373 (82.0)	6/373 (1.6)	61/373 (16.4)	352/428 (82.2)	12/428 (2.8)	64/428 (15.0)	0.468
Gentamicin	288/373 (77.2)	26/373 (7.0)	59/373 (15.8)	343/428 (80.1)	33/428 (7.7)	52/428 (12.2)	0.316
Tobramycin	309/373 (82.8)	1/373 (0.3)	63/373 (16.9)	357/428 (83.4)	3/428 (0.7)	68/428 (15.9)	0.644
Colistin	369/372 (99.2)	-	3/372 (0.8)	420/428 (98.1)	-	8/428 (1.9)	0.236
Ciprofloxacin	262/373 (70.3)	24/373 (6.4)	87/373 (23.3)	182/242 (75.2)	9/242 (3.7)	51/242 (21.1)	0.240

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Table 5. Comparison of antimicrobial resistance rates in *Klebsiella pneumoniae* isolated over the 2 study periods (2017-2019 and 2020-2022)

Antibiotic	2017-2019 (131 isolates)			2020-2022 (142 isolates)			*P*-value
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Amoxicillin/clavulanic acid	51/131 (38.9)	5/131 (3.8)	75/131 (57.3)	46/142 (32.4)	7/142 (4.9)	89/142 (62.7)	0.510
Piperacillin/tazobactam	53/131 (40.5)	9/131 (6.9)	69/131 (52.6)	26/62 (41.9)	3/62 (4.9)	33/62 (53.2)	0.858
Cefotaxime	55/131 (42.0)	-	76/131 (58.0)	71/142 (50.0)	-	71/142 (50.0)	0.224
Ceftriaxone	55/131 (42.0)	-	76/131 (58.0)	71/142 (50.0)	-	71/142 (50.0)	0.224
Ceftazidime	55/131 (42.0)	-	76/131 (58.0)	71/142 (50.0)		71/142 (50.0)	0.224
Cefepime	55/131 (42.0)	-	76/131 (58.0)	71/142 (50.0)	-	71/142 (50.0)	0.224
Aztreonam	55/131 (42.0)	-	76/131 (58.0)	72/141 (51.1)	-	69/141 (48.9)	0.145
Imipenem	66/131 (50.4)	-	65/131 (49.6)	81/142 (57.0)	-	61/142 (43.0)	0.277
Meropenem	66/131 (50.4)	-	65/131 (49.6)	81/142 (85.1)	-	61/142 (43.0)	0.277
Amikacin	62/131 (47.3)	1/131 (0.8)	68/131 (51.9)	91/142 (64.1)	9/142 (6.3)	42/142 (29.6)	<0.001
Gentamicin	106/131 (80.9)	2/131 (1.5)	23/131 (17.6)	105/142 (73.9)	5/142 (3.5)	32/142 (22.5)	0.312
Tobramycin	54/130 (41.5)	-	76/130 (58.5)	85/142 (59.9)	-	57/142 (40.1)	0.003
Colistin	111/131 (84.7)	-	20/131 (15.3)	110/142 (77.5)	-	32/142 (22.5)	0.164
Ciprofloxacin	56/131 (42.7)	-	75/131 (57.3)	28/63 (44.4)	-	35/63 (55.6)	0.870
Tetracycline	75/131 (57.2)	17/131 (13.0)	39/131 (29.8)	30/62 (48.4)	6/62 (9.7)	26/62 (41.9)	0.241
Tigecycline	99/126 (78.6)	11/126 (8.7)	16/126 (12.7)	41/60 (68.3)	8/60 (13.3)	11/60 (18.4)	0.316
TMP/SMX	62/130 (47.7)	-	68/130 (52.3)	39/62 (62.9)	-	23/62 (37.1)	0.063

TMP/SMX, trimethoprim-sulfamethoxazole.

piperacillin/tazobactam. The overall MDR *P. aeruginosa* was 13.5% while PDR reached 0.4%.

K. pneumoniae was the third most frequently isolated Gram-negative pathogen in our study. High rates of resistance were observed for the third and fourth generation cephalosporins, namely cefotaxime, ceftriaxone, ceftazidime, and cefepime and for the carbapenems, imipenem, and meropenem, ranging from 46.2% to 53.8%. High rates of resistance were also detected in ciprofloxacin (56.7%) and the complex of β-lactam/β-lactamase inhibitor antibiotics, such as amoxicillin/clavulanate (60.1%) and piperacillin/tazobactam (52.8%). Although resistance to amoxicillin/clavulanate, piperacillin/tazobactam, colistin, tetracycline, and tigecycline numerically increased, but not statistically significant, over the two three-year periods, resistance to cephalosporins, carbapenems, amikacin, tobramycin, and TMP/SMX decreased during the last three years (*P*=0.224, *P*=0.277, *P* <0.001, *P*=0.003, and *P*=0.063, respectively) (**Table 5**). The average prevalence of carbapenem resistant *K. pneumoniae* (CRKP) throughout the study period was 46.2%. Analyzing the carbapenem resistance trend over the two three-year periods, it was found there was a trend for decrease in resistance from 49.6% to 43% (*P*=0.277) (**Table 5**). Among the 126 CRKP isolates, 83 (65.9%), 30 (23.8%), 9 (7.2%), and 4 (4.2%) were positive for KPC, NDM, VIM, and OXA-48 enzymes, respectively.

The prevalence of MDR was notably high at 51.6% while PDR reached 1.1%. A significant decrease of MDR was detected during the last three years (*P* <0.001).

S. aureus was the most frequently isolated pathogen among Gram-positive bacteria. Resistance to penicillin was extremely high (79.8%). Resistance rates to oxacillin, erythromycin, and clindamycin were 37.2%, 33.4%, and 30.8%, respectively. Sixty-two percent of the methicillin-resistant strains were MDR. Resistance to erythromycin and clindamycin decreased from 35.7% and 34.1% over the period 2017–2019 to 31.6% and 28.3% over the years 2020–2022, respectively (*P* <0.001 and *P*=0.243, respectively) (**Table 6**). Low rates of resistance were detected in TMP/SMX (3.3%), gentamicin (2.8%), and rifampicin (0.9%). All isolates were susceptible to linezolid, daptomycin, tigecycline, teicoplanin, and vancomycin.

DISCUSSION

The present study provided an insight of the prevalence and antimicrobial resistance profiles of bacterial pathogens isolated from samples of patients with LRTIs between 2017 and 2022, in Crete, Greece. During the six-year period, a total of 3427 sputum and BALF specimens yielded a significant growth of bacteria. Consistent with other reports, the higher incidence of LRTIs found in males is mainly due to socio-economic and lifestyle related risk factors [12-15].

Table 6. Comparison of antimicrobial resistance rates in *Staphylococcus aureus* isolated over the 2 study periods (2017–2019 and 2020–2022)

Antibiotic	2017-2019 (185 isolates)			2020-2022 (240 isolates)			*P*-value
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Penicillin	38/185 (20.5)	-	147/185 (79.5)	48/240 (20)	-	192/240 (80.0)	0.903
Oxacillin	117/185 (63.2)	-	68/185 (36.8)	150/240 (62.5)	-	90/240 (37.5)	0.919
Gentamicin	179/185 (96.8)	-	6/185 (3.2)	234/240 (97.5)	-	6/240 (2.5)	0.770
Levofloxacin	142/183 (77.6)	2/183 (1.1)	39/183 (21.3)	176/240 (73.3)	9/240 (3.8)	55/240 (22.9)	0.202
Erythromycin	77/185 (41.6)	42/185 (22.7)	66/185 (35.7)	53/240 (22.1)	111/240 (46.3)	76/240 (31.6)	<0.001
Clindamycin	122/185 (65.9)	-	63/185 (34.1)	172/240 (71.7)	-	68/240 (28.3)	0.243
Linezolid	185/185 (100)	-	0/185	240/240 (100)	-	0/240	NA
Daptomycin	185/185 (100)	-	0/185	240/240 (100)	-	0/240	NA
Teicoplanin	185/185 (100)	-	0/185	240/240 (100)	-	0/240	NA
Vancomycin	185/185 (100)	-	0/185	240/240 (100)	-	0/240	NA
Tetracycline	165/185 (89.2)	-	20/185 (10.8)	218/240 (90.8)	-	22/240 (9.2)	0.624
Tigecycline	181/181 (100)	-	0/181	240/240 (100)	-	0/240	NA
Rifampicin	181/185 (97.8)	1/185 (0.6)	3/185 (1.6)	235/240 (97.9)	4/240 (1.7)	1/240 (0.4)	0.254
TMP/SMX	179/185 (96.8)	-	6/185 (34.3)	232/240 (96.7)	-	8/240 (3.3)	1.000

NA, not applicable; TMP/SMX, trimethoprim-sulfamethoxazole.

Among the 4,008 pathogens, 86.3% were Gram-negative bacteria and 13.7% were Gram-positive bacteria. The SENTRY Program found that Gram-negative bacteria represented 76.3%, 88.6%, and 69.1% of organisms isolated from respiratory samples in Western Europe (W-EU), Eastern Europe (E-EU) and United States (US)-of patients with pneumonia, during 2016-2019 [16]. Monomicrobial growth was detected in 74.3% of cases and polymicrobial in 25.7 of them. Our results compare favourably with a recent Italian study in which 74.7% of cases were monomicrobial and 25.1% polymicrobial [12].

The most prevalent bacterial pathogens found in this study were *A. baumannii*, *P. aeruginosa, K. pneumoniae,* and *S. aureus*, which is consistent with other studies [12, 17, 18]. Isolation rates of *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* were significantly higher in ICUs patients compared to those in the respiratory ward, similar to the findings of two Chinese hospitals [19, 20]. *A. baumannii* and *P. aeruginosa* were more prevalent during summer, *K. pneumoniae* was more common during autumn, while for *S. aureus* higher incidence was noted during winter [21, 22].

Antimicrobial susceptibility of *A. baumannii* showed high resistance rates to most antibiotics, except for tigecycline. According to the European Center for Disease prevention and Control on antimicrobial resistance in Europe, 88.6% of *Acinetobacter* invasive isolates in Greece were reported to have combined resistance to carbapenems, aminoglycosides, and fluoroquinolones in 2022 [23]. A recent nationwide surveillance of antimicrobial resistance conducted among 19 Greek hospitals, reported that *A. baumannii* resistance rates to meropenem and colistin were 98.9% and 84.5%, respectively [24]. *A. baumannii* has the propensity to develop rapid resistance. Additionally, it forms biofilms on medical devices, especially ICU ventilators [25]. *A. baumannii* is one of the leading causes of ventilator-associated pneumonia (VAP) globally, especially in Asia, Latin America, and the Middle East [26]. MDR *A. baumannii* strains are now prevalent worldwide. In our study MDR *A. baumannii* isolates reached 91%. The ICU mortality of MDR *A. baumannii*-caused VAP has been reported to be as high as 84.3% [27].

P. aeruginosa represented 20% of the bacterial pathogens isolated from respiratory samples of patients with LRTIs. This isolation rate is in line with that reported in W-EU during 2016-2019 [16]. *Pseudomonas* spp. are the second leading cause of nosocomial pneumonia in the US, causing 19% of hospital-acquired pneumonia and 21.4% of VAP [28]. *P. aeruginosa* susceptibility to piperacillin/tazobactam was 69.2%, lower to that detected among W-EU and E-EU (76.1% and 75.4%, respectively [16]. Colistin was active against 98.6% of *P. aeruginosa* isolates, similar to 99.3% in E-EU and 99.7% in the US [16]. Non-susceptibility to meropenem decreased from 35.7% during 2017-2019 to 33.6% over the period 2020-2022. A previous study from the same area, reported meropenem resistance for 38.4% of the 912 *P. aeruginosa* strains isolated from respiratory samples [29].

K. pneumoniae is the third most frequently isolated Gram-negative pathogen from LRTIs. It is most commonly associated with community-acquired pneumonia and VAP in ICUs [30]. In the present study, *K. pneumoniae* exhibited high rates of resistance to carbapenems

attributed to the production of carbapenemases, with KPC being the most prevalent. Greece is an endemic area for CRKP and its increased prevalence in recent years constitute a great public health threat. Carbapenem resistance among invasive *K. pneumoniae* has increased from 63.9% in 2018 to 72% in 2022 [23]. In 2022, the European Centre for Disease Prevention and Control and the National Public Health Organization in Greece published the results of their study, issuing a warning about the rapid spread of CRKP in Greek hospitals [31].

In the present study, LRTI associated with *S. aureus* was found to be 10.6%. It was also observed that all strains were susceptible to vancomycin, teicoplanin, linezolid, tigecycline, and daptomycin. *S. aureus* is a major pathogen of hospital-acquired pneumonia associated with significantly higher morbidity and mortality, much more than other hospital-associated pneumonias [32]. Methicillin-resistance was detected in 37.2% of the cases. This is slightly lower to that found in a national surveillance study conducted in Greece between 2012 and 2016 which found that 39% of *S. aureus* isolates were methicillin-resistant *S, aureus* (MRSA) [33]. In Greece, overuse and misuse of antibiotics have driven methicillin-resistance in *S. aureus* [34]. MRSA is now the major cause of hospital-associated infection, often causing VAP as a complication of intensive care.

The present study has two limitations. First, the study was conducted on a single geographical area, so the present findings are not representative of the epidemiology of LRTIs of the whole country, since the distribution of pathogens and antimicrobial resistance may fluctuate among geographic regions, even in different areas of the same country. Second, detailed clinical information on the patients' clinical characteristics and outcome was not available.

This study illustrates the prevalence and the antimicrobial resistance trends of bacterial pathogens associated with LTRIs, highlighting their high resistance and multi-resistance to antimicrobial agents. Continued monitoring of local microbial prevalence and antimicrobial resistance is of paramount importance to establish updated guidelines to optimize empiric treatment of LRTIs.

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