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ORIGINAL ARTICLE

Bacterial profile and their antimicrobial resistance patterns among patients with community-acquired pneumonia in southwestern Iran

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ABSTRACT

Background and Objectives: Community-acquired pneumonia (CAP) is one of the most common life-threatening infections, occurring in the community or within the first 48 hours of a patient's hospitalization. The present study aimed to investigate the frequency of pathogenic bacteria and their antibiotic resistance pattern in the sputum of patients with community-acquired pneumonia in Yasuj from 2018 to 2019.

Materials and Methods: In the present study, 128 patients with CAP were included. Under aseptic conditions clinical samples including sputum collected from each patient were sent to the Microbiology Laboratory. Specific culture media and biochemical tests were used to identify the bacteria. Antimicrobial resistance patterns of the isolates were examined by disc diffusion. DNA was extracted from sputum using the phenol-chloroform method. The PCR method was used for the molecular detection of bacteria. Data were analyzed using SPSS software version 22 and the chi-square test.

Results: The most common clinical symptoms in patients were sputum (68.8%), fever (64.1%), shortness of breath (60.2%), cough (50.8%), and chest pain (24.2%). A total of 133 bacteria were identified by culture and 117 bacteria by PCR. In the current study, the most prevalent organisms were *Streptococcus pneumoniae* (24.1%), *Hemophilus influenzae* (18%), *Staphylococcus aureus* (13.5%), and *Moraxella catarrhalis* (11.4%). Antibiogram test showed that most of the Gram-negative bacteria were resistant to levofloxacin (22.6%), rifampin (20.8%) and ceftriaxone (17%), and the highest resistance rate to clindamycin (43.1%), ciprofloxacin (43.1%) and amoxicillin (41.4%) were detected in the Gram-positive bacteria. Cefepime was the most effective antibiotic against Gram negative bacteria.

Conclusion: *S. pneumoniae* was the most prevalent bacteria identified by culture and PCR methods in patients with CAP, indicating an important role of this bacterium in the pathogenesis of CAP. According to the results, cefepime can be used to treat patients with CAP with Gram-negative bacteria. In the present study, *S. pneumoniae*, *S. aureus*, *P. aeruginosa*, *H. influenzae*, *M. catarrhalis*, and *K. pneumoniae* have been isolated from the CAP patient population with varying frequencies. This is consistent with various studies in different parts of the world.

Keywords: Community-acquired pneumonia; Pathogenic bacteria; Antibiotic susceptibility pattern

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INTRODUCTION

Community-acquired pneumonia (CAP) is an acute infection of the lower respiratory tract infection and is the most prevalent type of lung infection occurring outside of the hospital or within the first 48 hours of admission. CAP is one of the most prevalent reasons for hospitalization among infectious diseases worldwide, leading to high mortality rates and enormous healthcare expenditures (1-3). CAP is estimated to affect 9 to 14 per 1,000 people yearly, with 30 to 46% of patients requiring hospitalization (4-6). CAP mortality among hospitalized patients has been estimated 8% to 14% in the United States and 7.3% in Asian countries (7). Identifying and treating these patients is a public health system concern. Nearly 9% to 14% of all hospitalized patients required ICU hospitalization. According to reports, people, who require ICU care, have a 24% death rate (8, 9). The main signs and symptoms of CAP are including respiratory symptoms (cough, sputum, shortness of breath, and chest pain), general infection symptoms (fever, hypothermia, weakness, circulatory symptoms, and impaired consciousness), tachypnea, tachycardia, hypotension, and focal hearing impairment. Whereas these signs are not sensitive enough or specific for final diagnosis, hence confirmatory tests, such as chest imaging, is indicated (10, 11). In older age, viral respiratory infections, smoking, alcohol abuse, and chronic diseases (chronic obstructive pulmonary disease, asthma, bronchiectasis, congestive heart failure, diabetes, and immunosuppressive diseases) are the primary risk factors for CAP (12, 13). In general, Pneumonia can be diagnosed using a combination of clinical, physical, radiological, and microbiological criteria (14). Microorganisms play an important role in CAP, including the most important typical bacteria such as *Streptococcus pneumoniae, Hemophilus influenzae, Moraxella catarrhalis* and *Staphylococcus aureus.* These bacteria can grow in culture media and are stained using the Gram stain method. Another group does not easily grow in culture media and cannot be stained with Gram stain, such as *Legionella pneumophila, Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *C. psittaci* (15, 16). Despite improvements in microbiological diagnostic methods, the definite diagnosis of CAP-causing bacteria remains a challenging issue. Besides, collecting adequate and uncontaminated sputum samples from the lung is another problem that can lead to false-positive results (5, 17). Molecular method, such as polymerase chain reaction (PCR), has improved the detection of numerous bacterial and viral infections associated with CAP and has the advantage of detecting respiratory pathogens after antibiotic therapy (18, 19). This is because there is little information about the CAP-causing bacteria in southwest Iran. The aim of this study was to identify the bacteria and their antimicrobial resistance patterns in patients with CAP at a teaching hospital in Yasuj, Iran.

MATERIALS AND METHODS

This cross-sectional study was performed on patients with CAP admitted to the teaching hospital (internal medicine and infectious disease wards) affiliated with Yasuj University of Medical Sciences in southwest Iran over 14-month from 2018 to 2019. A total of 128 CAP patients ranging age from 18 to 88 years with a mean age of 57.92 years participated in this study.

The inclusion criteria were as follows: 1) age >15 years; 2) patients with community-acquired pneumonia or within the first 48 hours of hospitalization; and 3) patients based on clinical signs (Having at least one or two of the following characteristics: fever, leukopenia, leukocytosis, purulent secretion of lungs, cough and sputum). and radiological findings. All of the patients visited by a specialist in internal medicine and infectious diseases. First-morning sputum samples were collected from the patients' lungs after a deep cough, the sputum was placed in a sterile container, and transported to the microbiology laboratory for 2 hours. Sputum samples from patients with community-acquired ventilator-associated pneumonia were collected under aseptic conditions from the lower lung. In the microbiology lab, the collected sputum samples were divided into two parts. One part of the sputum is preserved in sterile microtubes at -20°C for molecular purposes. The other part is used for microbial culture and identification of bacteria using standard biochemical tests. The following media were used for the identification of bacteria at the species level: Blood agar with 5% sheep blood (CONDA, Spain) was used for the initial isolation of *S. pneumoniae* and *S. aureus.* Chocolate agar containing clindamycin (1 µg/mL), bacitracin (300 µg/mL) and vancomycin (5 µg/mL) used for

H. influenzae in the presence of 5% carbon dioxide. Chocolate agar containing clindamycin (1 µg/mL), bacitracin (300 µg/mL), vancomycin (5 µg/mL) and acetazolamide was used for *M. catarrhalis* (20). All of the antibiotics powders used in culture media were purchased from Sigma-Aldrich company (Sigma-Aldrich, Germany). MacConkey agar (CONDA, Spain) was used for Gram-negatives (*P. aeruginosa* and *K. pneumonia*). Demographic characteristics including age, sex, and clinical manifestations were collected. Prior sampling, from of each individual informed consent was obtained.

Antibiotic susceptibility test. We used antibiogram test according to the Clinical and Laboratory Standards Institute (CLSI) guideline using the disc agar diffusion method on Mueller-Hinton agar (CONDA, Spain), using the following antibiotics: cefepime (30 µg), ceftriaxone (30 µg), Azithromycin (15 µg), imipenem (10 µg), ceftazidime (30 µg), amikacin (30 µg), Clindamycin (2 µg), tetracycline (30 µg), clarithromycin (15 µg), ciprofloxacin (5 µg) (BD-BBL Company, USA). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as quality control.

Polymerase chain reaction (PCR) and multiplex PCR. The DNA from sputum samples was extracted using the phenol-chloroform method (immediately after sample homogenization, 500 µl of lysis buffer was added to the sample, incubate for one hour at laboratory temperature, then 12.5 µl of proteinase K was added to it, and incubated for 14 hours at 55°C. Thereafter 700 µl of Phenol: Chloroform: Isoamyl Alcohol 25:24:1 was added and incubated for one hour at room temperature and centrifuged at 12,000 rpm for 5 minutes. The supernatant was transferred to a new vial with 70% ethanol and after precipitation of DNA, repeated with 100% ethanol then draining the ethanol completely, after drying the DNA precipitate, it was dissolved in 100 µl of distilled water and stored as a DNA template at -20°C for further purposes. Bacteria were identified using specific primers (Table 1) by PCR and Multiplex PCR (20-23). The presence of *S. pneumoniae, H. influenzae,* and *M. catarrhalis* in clinical specimens was determined using multiplex PCR. The standard strains of *S. pneumoniae* ATCC49619, *H. influenzae* ATCC49247, and *M. catarrhalis* ATCC25238 were used as positive controls.

The multiplex PCR reaction condition with following program was used for detection of bacteria. Primary denaturation at 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 45 s, 58°C for 45 s for *H. influenzae, M. catarrhalis* and *S. pneumoniae,* 55°C for 45 s for *S. aureus* and extension at 72ºC for 1 min. The final extension was continued at 72ºC for 5 min and was performed in a thermocycler (Bio-Rad, T100, USA).

For detection of *P. aeruginosa* and *K. pneumoniae,* the program was as follows: initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 45 s, annealing (30 s at 59°C for *oprL*, 60 s at 57°C for *ureD*) and extension at 72°C for 1 min and was followed by a final cycle of extension for 5 min at 72°C. PCR products were analyzed by gel electrophoresis on 1% agarose gel at 95 V for 50 min and after staining with DNA safe Stain, gel documentation system (Major Sciences, Taiwan) used for visualization of PCR products.

RESULTS

Among the 128 CAP patients 77 (60.2%) were male and 51 (39.8%) were female. Sputum, fever, shortness of breath, cough, and chest discomfort were found in 68.8%, 64.1%, 60.2%, 50.8%, and 24.2% out of the 128 patients, respectively. The microorganisms were identified among 83 (64.8%) and 95 (74%) of patients using culture and PCR methods, respectively (Table 2).

A total of 133 bacteria were identified from patients using culture method. *S. pneumoniae* was the most prevalent pathogen and was isolated from 32 (24.1%) of patients, *H. influenzae,* other streptococci, *S. aureus,* and *M. catarrhalis* isolated from 24 (18%), 22 (16.5%), 18 (13.5%), and 15 (11.4%) patients respectively (More details in the Table 3).

Then, of 128 patients tested, 95 were PCR positive. A total of 117 bacteria were detected by PCR. *S. pneumoniae* was the major pathogen detected in 37 (31.6%) of patients. The detection rates of *H. influenzae, M. catarrhalis,* and *S. aureus* were 28.1% (n=33), 22.2% (n=26), and 11.1% (n=13) respectively.

There was no statistically significant difference between bacteria isolated by culture and bacteria detected by PCR (Table 3). In the present study sputum (68.8%), fever (64.1%), shortness of breath (60.2%), cough (50.8%) and chest pain (24.2%) were the most prevalent clinical presentation.

Antibiogram test showed that most of the Gram-neg-

Table 1. The oligonucleotides sequences of primers used in this study.

Target Gene	Primer Sequence (5' →3')	Amplicon Length, bp
Common primer	5′- CTA CGC ATT TCA CCG CTA CAC-3′	
H. influenza	CGT ATT ATC GGA AGA TGA AAG TGC-3′	525
M. catarrhalis	5′- CCC ATA AGC CCT GAC GTT AC-3′	237
S. pneumonia	5′- AAG GTG CAC TTG CAT CAC TAC C-3′	484
S. aureus (nucA)	F 5′- CTG GCA TAT GTA TGG CAA TTG TT - 3′	670
	R 5′- TAT TGA CCT GAA TCA GCG TTG TCT - 3′	
K. pneumoniae (ure-D)	F 5'-CCC GTT TTA CCC GGA AGA AG-3	243
	R 5'-GGA AAG AAG ATG GCA TCC TGC-3'	
P. aeruginosa (opr-L)	F 5′-ATGGAAATGCTGAAATTCGGC-3	504
	R 5′-CTTCTTCAGCTCGACGCGACG-3′	

Table 2. frequency of bacteria using culture and PCR

Methods	Culture + PCR+	Culture + PCR -	Culture - PCR+
Frequency	60%, 77 cases	4.7%, 6 cases	14%, 18 cases

Table 3. Identification and detection of bacteria using PCR and culture methods

Bacteria	Identification method		P
	PCR (N=117)	Culture (N=133)	
S. aureus	13 (11.1%)	18 (13.5%)	0.18
S. pneumoniae	37 (31.6%)	32 (24.1%)	0.5
H. influenzae	33 (28.1%)	24 (18%)	0.071
M. catarrhalis	26 (22.2%)	15 (11.4%)	0.15
P. aeruginosa	4 (3.5%)	8 (6%)	0.219
K. pneumoniae	4 (3.5%)	6 (4.5%)	0.625
Other streptococci	-	22 (16.5%)	-
Coagulase-negative Staphylococci	-	8 (6%)	-

ative bacteria were resistant to levofloxacin (22.6%), rifampin (20.8%) and ceftriaxone (17%) (Table 4) and the highest resistance rate to clindamycin (43.1%), ciprofloxacin (43.1%) and amoxicillin (41.4%) were detected in the Gram-positive bacteria (Table 5).

Table 4. Antimicrobial resistance pattern of Gram-positive bacteria.

Antibiotics	*S. aureus*	S. pneumoniae
Azithromycin	50%	21.9%
tetracycline	42.3%	34.4%
Clindamycin	46.2%	40.6%
Amoxicillin	50%	34.4%
Amikacin	50%	12.5%
Ciprofloxacin	61.5%	28.1%
doxycycline	38.5%	28.1%
Clarithromycin	46.2%	34.4%

DISCUSSION

Community-acquired pneumonia is one of the most common life-threatening infections. This pneumonia develops in the community or during the first 48 hours of hospitalization and has the highest mortality rate in developing countries. Currently, Sputum, fever, and shortness of breath were the most prevalent clinical signs. Batool et al. reported that sputum was more prevalent which is similar to the present study (24). El-Sokkary et al. and Cilloniz et al. found that fever and cough to be common clinical symptoms

Table 5. Antimicrobial resistance pattern of Gram-negative bacteria isolated in this study.

Antibiotics	*H. influenza*	*M. catarrhalis*	*P. aeruginosa*	*K. pneumoniae*
levofloxacin	20.8%	6.7%	50%	66.7%
Amikacin	16.6%	0	25%	-
Clarithromycin	25%	6.7%	-	-
Erythromycin	8.3%	-	50%	33.3%
Ceftriaxone	4.2%	0	50%	50%
Imipenem	0	0	50%	66.7%
Cefepime	0	0	37.5%	66.7%
Ceftazidime	16.6%	0	37.5%	33.3%
Ciprofloxacin	-	6.7%	50%	33.3%

(25, 26). While cough and chest pain were less common in the present study in comparison with Batool et al. Cilloniz et al., El Sokkary et al., and Lupisanet et al. studies (1, 24, 25, 27). Differences in clinical symptoms among different studies may be related to various factors, such as patients' age and their immunological status, duration of disease involvement, physical condition (such as obesity) and the type of organisms that cause CAP (typical or atypical).

The prevalence of Gram-positive bacteria was more common than Gram-negative bacteria which is consistent with previous studies from Iran (28), the Philippines (1), Ethiopia (29), and Saudi Arabia (24). Similar to other studies (30-33), *S. pneumoniae* was the most prevalent bacteria in the current study, indicating the important role of *S. pneumoniae* in the pathogenesis of CAP. In this study, *H. influenzae* was the second most frequent organism identified in 18% of the study subjects. This is consistent with the report of Lupisan et al. (1), comparing to Costa et al., Temesgen et al. and Luan et al. (30, 34, 35). *S. aureus* was identified in 13.5% of patients in the current study, which was higher than the reports from the Philippines and Egypt (1, 25). This organism can colonize the nose and nasopharynx and, probably, one of the reasons for the role of this organism in CAP is related to the colonization of the organism in these areas.

M. catarrhalis can cause infections in the upper and lower respiratory tract, making it an important and common upper respiratory microbial pathogen after *S. pneumoniae* and *H. influenzae*. In this study *M. catarrhalis* was isolated from 11.4% of patients, which was higher than studies by Costa et al, Lupison et al., and Blejan et al. investigations (1, 35, 36). *P. aeruginosa*, another pathogen, was detected in only 6% of patients, which was less common than studies (10-12%) from other countries (24, 27, 34, 36). In respiratory infections, this bacterium's pathogenicity is mediated by bacterial adhesion to host epithelial cells. Adhesins are one of the most prominent *P. aeruginosa* virulence factors in respiratory infections. Adhesion and colonization, using flagella, polysaccharide capsules, and fimbriae are the most important factors in developing *P. aeruginosa* respiratory infections. *K. pneumoniae* was isolated in only 4.5% of patients, and it is the lowest prevalence in the current study. Luan et al. (34) reported *K. pneumoniae* was the most common isolated organism. In general, there were differences in bacterial prevalence among different studies. Factors such as differences in clinical management of the diseases, diversity of the study population, patient's immune system status, and variation in a public health surveillance system can also be effective.

In the present study, 61.5%, 50%, 50%, 50%, and 38.5% of *S. aureus* isolates were resistant to ciprofloxacin, azithromycin, amoxicillin, amikacin, and doxycycline, respectively. Batool et al. found higher resistant rate among *S. aureus* isolates against amikacin (90%), ciprofloxacin (80%), azithromycin (70%) and doxycycline (60%) while, in a study by Temesgen et al. the resistance rate to ciprofloxacin (29.2%), and doxycycline (41.7%) were lower than in our study (24, 30). The resistance rate to clindamycin, doxycycline, ciprofloxacin, azithromycin, and amikacin and among *S. pneumoniae* were 40.6%, 28.1%, 28.1%, 21.9% and 12.5%, respectively. In contrast to our study, El-Sokkary et al. showed the resistant rate to ciprofloxacin (65.2%), azithromycin (47.8%), and clindamycin (87%) were much higher, and also Batool et al. showed higher resistant rate in comparison to our study, including amikacin (92%), doxycycline (80.7%), ciprofloxacin (61.5%), and azithromycin

(84.6%) (24, 25). In *H. influenzae* strains the resistant rate to clarithromycin, levofloxacin, amikacin and ceftazidime were 25%, 20.8%, 16.6%, and 16.6%, respectively. In a study by Batool et al. the resistant rate of *H. influenzae* against ceftazidime (66.6%), amikacin (83.3%), levofloxacin (91.6%), and clarithromycin (41.6%) were much higher than in our study (24). In the current study, *P. aeruginosa* isolates were resistant to ciprofloxacin (50%), ceftriaxone (50%), and ceftazidime (37.5%), while most of the isolates were sensitive to amikacin (75%) which were higher than the study by Temesgen et al. (30) showed that their *P. aeruginosa* isolates were resistance to ceftriaxone (23.6%), ciprofloxacin (21.1%), and ceftazidime (5.3%). Analysis of the antibiotic resistant rate in different studies showed that the rate of resistance differs between studies. This may be due to empirical use of antibiotics, different infection control policies in the geographical areas, duration of patient's hospitalization, and availability of medical instruments.

CONCLUSION

S. pneumoniae was the most prevalent bacteria identified by culture and PCR methods in patients with CAP, indicating the important role of this bacterium in pathogenesis of CAP. According to the results cefepime was the most effective antibiotic against Gram negative bacteria.

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REFERENCES

1. Lupisan S, Suzuki A, Macalalad N, Egos R, Sombrero L, Okamoto M, et al. Etiology and epidemiology of community-acquired pneumonia in adults requiring hospital admission: A prospective study in rural Central Philippines. *Int J Infect Dis* 2019; 80: 46-53.
2. Shoar S, Musher DM. Etiology of community-acquired pneumonia in adults: a systematic review. *Pneumonia (Nathan)* 2020; 12: 11.
3. Hassen M, Toma A, Tesfay M, Degafu E, Bekele S, Ayalew F, et al. Radiologic Diagnosis and Hospitalization among Children with Severe Community Acquired Pneumonia: A Prospective Cohort Study. *Biomed Res Int* 2019; 2019: 10.1155/2019/6202405.
4. Kolditz M, Ewig S. Community-acquired Pneumonia in adults. *Dtsch Arztebl Int* 2017; 114: 838-848.
5. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, et al. Community-acquired Pneumonia requiring hospitalization among U.S. adults. *N Engl J Med* 2015; 373: 415-427.
6. Myint PK, Kwok CS, Majumdar SR, Eurich DT, Clark AB, España PP, et al. The international community-acquired Pneumonia (CAP) Collaboration Cohort (ICCC) study: rationale, design and description of study cohorts and patients. *BMJ Open* 2012; 2(3): e001030.
7. Song J-H, Oh WS, Kang C-I, Chung DR, Peck KR, Ko KS, et al. Epidemiology and clinical outcomes of community-acquired pneumonia in adult patients in Asian countries: a prospective study by the Asian network for surveillance of resistant pathogens. *Int J Antimicrob Agents* 2008; 31: 107-114.
8. Cavallazzi R, Furmanek S, Arnold FW, Beavin LA, Wunderink RG, Niederman MS, et al. The burden of community-acquired pneumonia requiring admission to ICU in the United States. *Chest* 2020; 158: 1008-1016.
9. Aydoğdu M, Ozyilmaz E, Aksoy H, Gürsel G, Ekim N. Mortality prediction in community-acquired pneumonia requiring mechanical ventilation; values of pneumonia and intensive care unit severity scores. *Tuberk Toraks* 2010; 58: 25-34.
10. Garin N, Marti C, Scheffler M, Stirnemann J, Prendki V. Computed tomography scan contribution to the diagnosis of community-acquired pneumonia. *Curr Opin Pulm Med* 2019; 25: 242-248.
11. Ebell MH, Chupp H, Cai X, Bentivegna M, Kearney M. Accuracy of signs and symptoms for the diagnosis of community-acquired Pneumonia: a meta-analysis. *Acad Emerg Med* 2020; 27: 541-553.
12. Almirall J, Serra-Prat M, Bolíbar I, Balasso V. Risk factors for community-acquired Pneumonia in adults: a systematic review of observational studies. *Respiration* 2017; 94: 299-311.
13. Muthumbi E, Lowe BS, Muyodi C, Getambu E, Gleeson F, Scott JAG. Risk factors for community-acquired pneumonia among adults in Kenya: a case–control study. *Pneumonia (Nathan)* 2017; 9: 17.
14. Harrison TR. Medicina interna de Harrison. Medicina interna de Harrison 2017. p. 665
15. Kumar KJ, Ashok Chowdary KV, Usha HC, Kulkarni M, Manjunath VG. Etiology of community acquired pneumonia among children in India with special reference to atypical pathogens. *Lung India* 2018; 35: 116-120.

16. Ojuawo OB, Desalu OO, Fawibe AE, Ojuawo AB, Aladesanmi AO, Opeyemi CM, et al. Clinical and microbiological profile of adult inpatients with community acquired pneumonia in Ilorin, North Central, Nigeria. *Afr Health Sci* 2020; 20: 1655-1668.
17. Musher DM, Roig IL, Cazares G, Stager CE, Logan N, Safar H. Can an etiologic agent be identified in adults who are hospitalized for community-acquired pneumonia: results of a one-year study. *J Infect* 2013; 67: 11-18.
18. Bartlett JG. Diagnostic tests for agents of community-acquired pneumonia. *Clin Infect Dis* 2011; 52 Suppl 4: S296-304.
19. Johansson N, Kalin M, Giske CG, Hedlund J. Quantitative detection of *Streptococcus pneumoniae* from sputum samples with real-time quantitative polymerase chain reaction for etiologic diagnosis of community-acquired pneumonia. *Diagn Microbiol Infect Dis* 2008; 60: 255-261.
20. Sabz G, Moradi S, Sharifi A, Naghmachi M, Taheripour Sisakht M, Khoramrooz SS. Identification and detection of pathogenic bacteria in adenoid tissue of adenoidectomized children: emergence of *Staphylococcus aureus* as the most prevalent pathogen. *Jundishapur J Microbiol* 2020; 13(1): e95445.
21. Askarinia M, Ghaedi M, Manzouri L, Khoramrooz SS, Sharifi A, Ghalamfarsa G, et al. The effect of Cu-BP-DCA-Ty on antibacterial activity and the expression of *mecA* gene in clinical and standard strains of methicillin-resistant *Staphylococcus aureus. Jundishapur J Microbiol* 2018; 11(3): e60680.
22. Zamani A, Yousefi Mashouf R, Ebrahimzadeh Namvar AM, Alikhani MY. Detection of *magA* gene in *Klebsiella* spp. isolated from clinical samples detection of *magA. Iran J Basic Med Sci* 2013; 16: 173-176.
23. Najafimosleh M, Rashnotaie S, Ghaznavi Rad E, Abtahi H, Taleie G. Designing of the specific DNA primers for detection of the *exoA, oprL* and *algD* pathogenicity genes for rapid diagnosis of *Pseudomonas aeruginosa*. *Tehran Univ Med J* 2013; 71: 493-501.
24. Batool S, Almaghaslah D, Alqahtani A, Almanasef M, Alasmari M, Vasudevan R, et al. Aetiology and antimicrobial susceptibility pattern of bacterial isolates in community acquired pneumonia patients at Asir region, Saudi Arabia. *Int J Clin Pract* 2021; 75(2): e13667.
25. El-Sokkary RH, Ramadan RA, El-Shabrawy M, El-Korashi LA, Elhawary A, Embarak S, et al. Community acquired pneumonia among adult patients at an Egyptian university hospital: bacterial etiology, susceptibility profile and evaluation of the response to initial empiric antibiotic therapy. *Infect Drug Resist* 2018; 11: 2141-2150.
26. Cilloniz C, Martin-Loeches I, Garcia-Vidal C, San Jose A, Torres A. Microbial etiology of pneumonia: epidemiology, diagnosis and resistance patterns. *Int J Mol Sci* 2016; 17: 2120.
27. Cillóniz C, Ewig S, Polverino E, Marcos MA, Esquinas C, Gabarrús A, et al. Microbial aetiology of community-acquired pneumonia and its relation to severity. *Thorax* 2011; 66: 340-346.
28. Hashemi SH, Soozanchi G, Jamal-Omidi S, Yousefi-Mashouf R, Mamani M, Seif-Rabiei M-A. Bacterial aetiology and antimicrobial resistance of community-acquired pneumonia in the elderly and younger adults. *Trop Doct* 2010; 40: 89-91.
29. Regasa B, Yilma D, Sewunet T, Beyene G. Antimicrobial susceptibility pattern of bacterial isolates from community-acquired pneumonia patients in Jimma University specialized hospital, Jimma, Ethiopia. *Saudi J Health Sci* 2015; 4: 59-64.
30. Temesgen D, Bereded F, Derbie A, Biadglegne F. Bacteriology of community acquired pneumonia in adult patients at Felege Hiwot Referral Hospital, Northwest Ethiopia: a cross-sectional study. *Antimicrob Resist Infect Control* 2019; 8: 101.
31. Para RA, Fomda BA, Jan RA, Shah S, Koul PA. Microbial etiology in hospitalized North Indian adults with community-acquired pneumonia. *Lung India* 2018; 35: 108-115.
32. Bjarnason A, Westin J, Lindh M, Andersson L-M, Kristinsson KG, Löve A, et al. Incidence, etiology, and outcomes of community-acquired pneumonia: a population-based study. *Open Forum Infect Dis* 2018; 5: ofy010.
33. Chi H, Huang Y-C, Liu C-C, Chang K-Y, Huang Y-C, Lin H-C, et al. Characteristics and etiology of hospitalized pediatric community-acquired pneumonia in Taiwan. *J Formos Med Assoc* 2020; 119: 1490-1499.
34. Luan Y, Sun Y, Duan S, Zhao P, Bao Z. Pathogenic bacterial profile and drug resistance analysis of community-acquired pneumonia in older outpatients with fever. *J Int Med Res* 2018; 46: 4596-4604.
35. Costa M, Cipriano A, Santos F, Valdoleiros S, Furtado I, Machado A, et al. Clinical profile and microbiological aetiology diagnosis in adult patients hospitalized with community-acquired pneumonia. *Pulmonology* 2022; 28: 358-367.
36. Blejan IE, Diaconu CC, Letiția A, Arsene DIU, Ghica M, Drăgănescu D, et al. Antibiotic resistance in community-acquired pneumonia. A Romanian perspective. *FARMACIA* 2020; 68: 512-520.