Original Article

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Microbial Characteristics of Lower Respiratory Tract Infections in Patients Referred from Primary Care Hospitals

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Abstract:

Objective: This study investigated the microbiological characteristics of severe lower respiratory tract infection patients not respond to initial treatment, and were transferred to a tertiary-level hospital.

Material and Methods: This was a multicenter, prospective study conducted across four hospitals: Cho Ray, Pham Ngoc Thach, Gia Dinh People's Hospital, and Can Tho Central General Hospital. Sputum specimens were collected shortly after admission and subjected to culture and real-time PCR testing.

Results: Out of the 252 patients, 170 (67.4%) met the criteria for analysis and identification of pathogenic microorganisms. The most frequently isolated pathogens were *Streptococcus pneumoniae (S. pneumonia)* and *Haemophilus influenzae (H. influenzae)*, comprising of 27.0% and 13.1%, respectively. Antibiotic susceptibility testing was conducted on 55 patients (32.3%). Among these cases, there were 16 instances of *S. pneumoniae*, 10 cases of *Staphylococcus aureus (S. aureus)*, 10 cases of *Pseudomonas aeruginosa (P. aeruginosa)*, 14 cases of gram-negative enteric bacteria, 2 cases of *H. influenzae*, 1 case of *Moraxella catarrhalis (M. catarrhalis)*, 1 case of *Enterococcus faecium*, and 1 case of *M. pneumoniae.* The

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antibiogram results revealed significant findings; including a 70% prevalence of extended-spectrum beta-lactamase and 90% occurrence of Methicillin-Resistant *Staphylococcus aureus*. The data also indicated high resistance rates; such as 90% resistance to erythromycin, 40-80% to ciprofloxacin, 30-80% to ceftazidime, and 30-40% to imipenem.

Conclusion: Pathogens associated with LRTIs referred from primary care hospitals encompass *S. pneumonia*, *H. influenza, and S. aureus*. At our facility, piperacillin, imipenem, amikacin, vancomycin, and linezolid emerged as the most effective antibiotics for addressing these LRTIs.

Keywords: antibiotic, culture, lower respiratory tract infections, pathogens, real-time PCR

Introduction

Lower respiratory tract infections (LRTIs) pose a significant global public health challenge, standing as a leading cause of mortality among individuals; spanning from children to adults worldwide[1]. In developing countries, the prevalence of LRTIs and pneumonia is markedly higher in developing countries, ranging between 20% and 30%, as compared to the lower rates of 3% to 4% observed in developed nations[2]. With an average population of 62.3 individuals per 10,000, annual hospital admissions for LRTIs ranged from 15 per 10,000 in the age range of 16–40 years to over 300 per 10,000 in the population over 79 years[2]. Community-acquired pneumonia (CAP) accounted for less than 37% of admissions, and 41.2% of individuals with pre-existing respiratory diseases. Remarkably, there exists a dearth of published reports elucidating the management or outcome of LRTI patients undergoing hospitalization within primary care facilities[2,3].

Acute respiratory tract infections are the major cause of primary care consultations[4] and unnecessary administration of antibiotics[5,6]. Across Europe, antibiotic prescriptions for acute respiratory tract infections range from 30% to 80%, with higher rates in southern Europe and lower rates in central and northern Europe; including Germany[7,8]. Antibiotic overuse exacerbates the challenge of antibiotic resistance; thereby, carrying substantial repercussions for global healthcare. These consequences encompass a diminished arsenal of effective treatments, prolonged hospital stays, heightened medical expenditures, and an increased mortality rate. Furthermore, the improper prescription of antibiotics is linked to the development of unnecessary adverse medication reactions[8].

This study aimed to investigate the microbiological characteristics of severe cases that exhibited inadequate responses to initial treatments that necessitated transfer to a higher-tier medical facility. The insights garnered from this investigation are poised to facilitate the selection of suitable empirical antibiotic treatments.

Material and Methods

This was a prospective, multicenter study involving four hospitals: Cho Ray Hospital, Pham Ngoc Thach Hospital, Gia Dinh People's Hospital, and Can Tho Central General Hospital; from 2016 to 2017. Ethical approval for the study was obtained from all four hospitals' Medical and Health Research Ethics Committees. The study adhered to the principles outlined in the Helsinki Declaration of 1964, and its subsequent revisions; ensuring the rights and well-being of the participants throughout the research endeavor.

Patients

We included individuals aged 18 years and older, presenting with lower respiratory tract infections (LRTIs), having been referred from primary care establishments; such as district or provincial hospitals. These patients were transferred to the recruiting sites after receiving a minimum

of 48 hours prior to treatment. The specific LRTI diagnoses of focus included: CAP or acute exacerbations of chronic obstructive pulmonary disease (AECOPD). gathered Data was gathered through patient interviews, review of their medical records, and conducted, comprehensive clinical assessments upon admission.

Microbiological test

All patients in the study had their sputum specimens collected immediately upon admission. The specimens were sent to a microbiology laboratory, where they underwent gram staining and microscopic examination under low power (x100). Specimens that were more than 25 leukocytes and less than 10 epithelial cells per low-power field"[9], would be selected for further quantitative culture and real-time Polymerase Chain Reaction (PCR) analysis. Microorganisms identified with a concentration of ≥10^5 colony-forming units (CFU) per milliliter by culture, and ≥10^5 copies per milliliter by multiplex real-time PCR were considered pathogenic.

An antibiotic susceptibility test was conducted using the dilution method on Mueller-Hinton agar for specimens that yielded consistent microbiological results, using culture and multiplex real-time PCR methods. The assessment of antibiotic sensitivity followed the standards set by the Clinical and Laboratory Standards Institute in 2015[10]. The same panel of antibiotics was used for all patients included in the study to determine their antibiotic susceptibility.

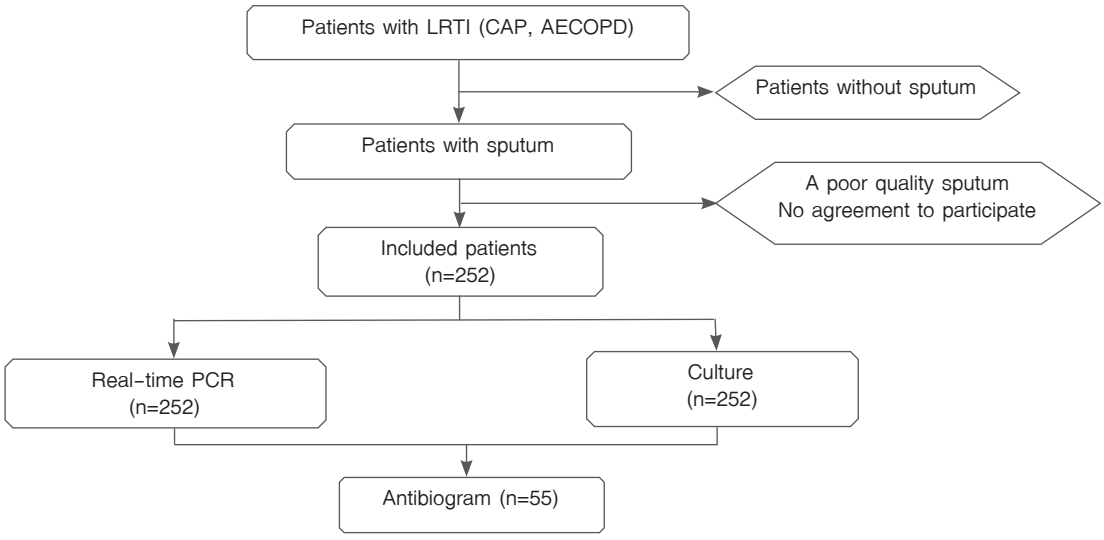
Statistical analysis

Descriptive data were presented using frequencies and percentages. All data analyses were performed using Statistical Package for the Social Science 22.0 software. Charts were created using Microsoft Excel 2010.

Results

Demographic and clinical characteristics

Out of the total 252 patients in the study (Figure 1), males accounted for 61.3%. The average age



CAP=community-acquired pneumonia, AECOPD=acute exacerbations of chronic obstructive pulmonary disease, PCR=polymerase chain reaction

Figure 1 A flow chart of the research process

of the patients was 68.9 years, with 68.7% being 65 years or older. Among the patients, 174 (69.1%) were diagnosed with CAP, while 78 (30.9%) had AECOPD. Most cases were classified as severe CAP (*Pneumonia* Severity Index (PSI) III-V) or moderate-severe exacerbations of chronic obstructive pulmonary disease. Additionally, a significant percentage of patients had co-morbidities (36.9%), reduced mobility (23.4%), and structural lung diseases (40.9%) (Table 1).

Table 1 Patient characteristics on admission

Characteristics	Number of patients (n=252)	Rate (%)
Gender		
Male	164	61.5
Female	88	43.9
Age (years)		
<65	79	31.3
≥65	173	68.7
Diagnosis		
AECOPD	78	30.9
CAP	174	69.1
COPD exacerbation severity		
Mild	0	0.0
Moderate	19	7.5
Severe	59	23.4
Severity of CAP (PSI)		
Grade I	0	0.0
Grade II	33	13.1
Grade III	64	25.4
Grade IV	66	26.2
Grade V	11	4.4
History of disease		
Comorbidity	93	36.9
Immunodeficiency	22	8.7
Reduced mobility	59	23.4
Home care	154	61.1
Structural lung diseases	103	40.9

CAP=community-acquired pneumonia, AECOPD=acute exacerbations of chronic obstructive pulmonary disease, PSI=Pneumonia severity index

Microbiological characteristics

In total, 170 samples (67.4%) met the criteria for analyzing and identifying for pathogenic microorganisms: microbiological classification results are presented in Table 2. These indicated that *Streptococcus pneumonia (S. pneumonia)* and *Haemophilus influenzae (H. influenzae)* were the most frequently isolated pathogens; accounting for 27.0% and 13.1%, respectively (Table 2).

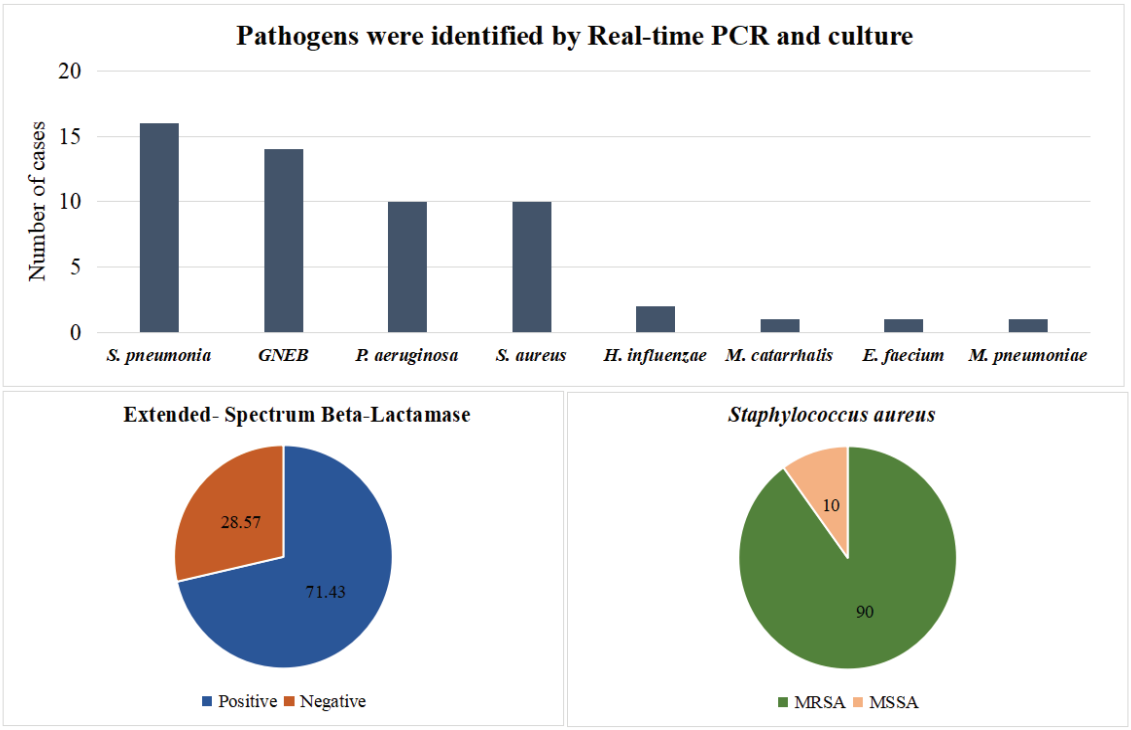
Antibiogram analysis was performed on 55 patients (32.3%). This encompassed 16 cases of *S. pneumoniae*, 10 cases of *Staphylococcus aureus (S. aureus)*, 10 cases of *Pseudomonas aeruginosa (P. aeruginosa)*, 14 cases of GNEB, 2 cases of *H. influenzae*, 1 case of *Moraxella catarrhalis (M. catarrhalis)*, 1 case of *Enterococcus faecium (E. faecium)*, and 1 case of *Mycoplasma pneumoniae* (Figure 2). Notably, among the *S. aureus* isolates, 90% exhibited MRSA characteristics (Figure 2). The antibiograms of *S. pneumoniae*, *P. aeruginosa*, and GNEB are depicted in Figure 3.

Table 2 Distribution of pathogenic bacteria isolated in this study

Microorganism	Number of patients (n=170)	Rate (%)
S. pneumoniae	68	27.0
S. aureus	10	4.0
M. catarrhalis	6	2.4
H. influenzae	33	13.1
M. pneumoniae	1	0.4
P. aeruginosa	11	4.4
K. pneumoniae	15	6.0
E. coli	7	2.8
Enterobacteriaceae	1	0.4
A. baumannii	10	4.0
E. faecium	4	1.6
M. tuberculosis	4	1.6
Total	170	100

Discussion

Bacteria isolated in LRTIs are typically extracellular, with the most commonly identified pathogens being *S. pneumoniae, H. influenzae, S. aureus*, and *M. catarrhalis*[3,11–13]. ESBL pathogens and *P. aeruginosa* are also frequently encountered[14–16]. The prevalence of these pathogens exhibits seasonal variations, as well as differences based on age, gender, and department[17–22]. According to



PCR=polymerase chain reaction, GNEB=gram-negative enteric bacteria, MRSA=methicillin-resistant staphylococcus aureus, MSSA=meticillin-sensitive staphylococcus aureus

Figure 2 Pathogens were identified by real-time PCR and culture

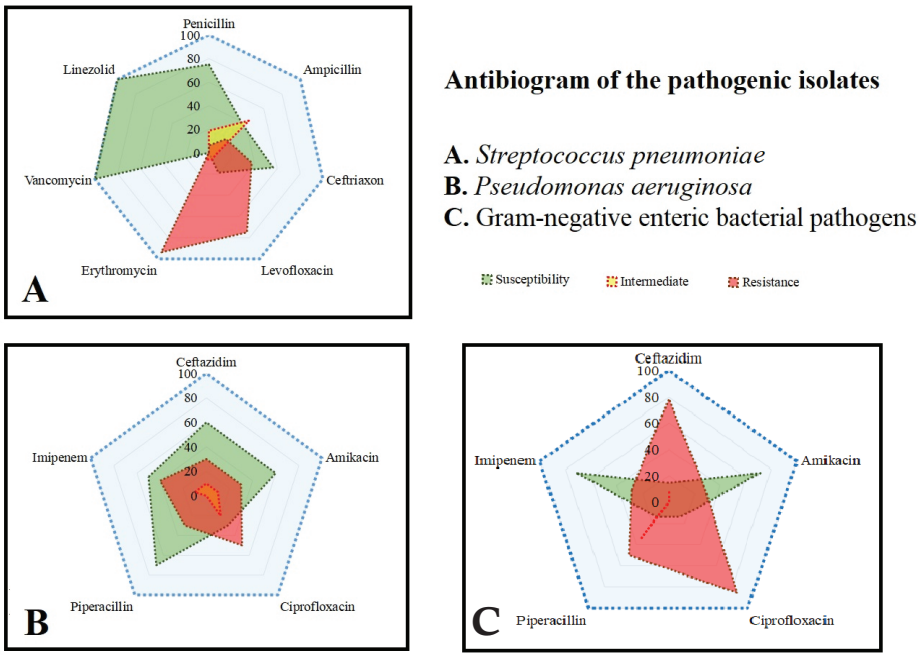


Figure 3 Antibiogram of the pathogenic isolates

updated data from 2005-2010, the European Respiratory Society and the European Society of Clinical Microbiology and Infectious Diseases reported *S. pneumoniae* and *H. influenzae* as the two most prevalent bacterial pathogens in CAP and AECOPD[18]. In 2021, a study by Biagio Santella analyzed 7,038 sputum samples obtained from hospitalized LRTIs patients using culture methods, the results indicated that 72.5% of the isolates were gram-negative, while only 24.5% were gram-positive. *Acinetobacter baumannii (A. baumannii)* was the most commonly identified pathogen among the Gram-negative group, while *S. aureus* predominated the Gram-positive group[19]. In 2015, a study analyzed 2,318 sputum samples from LRTIs, and found that *Klebsiella pneumonia (K. pneumonia), P. aeruginosa, Escherichia coli (E.coli), A. baumannii, S. aureus, Candida albicans, S. pneumoniae, and Streptococcus pyogenes* were the most common pathogens[20].

PCR has proven to be more effective than conventional methods in detecting infectious pathogens in patients with LRTIs[21,22]. PCR-based isolation techniques may also yield higher detection rates for *S. pneumoniae* and *H. influenzae* than conventional culture techniques[18,20,21]. In this study, the utilization of PCR for diagnosis resulted in a similar identification rate for *S. pneumoniae, H. influenzae*, and *M. catarrhalis*; as shown in Table 2, compared to other studies employing the same microbiological method. Additionally, it is noteworthy that the proportions of GNEB, *P. aeruginosa*, and *S. aureus* were elevated; as illustrated in Table 2.

Pathogens causing LRTIs exhibit significant resistance to various antibiotics, particularly gram-negative organisms[23,24]. The study sites observed a high prevalence of community-acquired LRTIs, with isolates demonstrating resistance to commonly used antibiotics; such as ampicillin, augmentin, ceftazidime, and tetracycline[19,25]. In antimicrobial susceptibility testing, *S. aureus* isolates displayed exceptional resistance to penicillin G (84.1%) and oxacillin (48.1%), but exhibited high sensitivity to tigecycline (100%) and linezolid (99.5%). *A. baumannii* isolates showed complete sensitivity to colistin, but resisted other antibiotics (95-99%). *K. pneumoniae* isolates exhibited significant resistance to cefotaxime (72.7%) and moderate sensitivity to gentamicin (54.3%). *P. aeruginosa* demonstrated resistance to ciprofloxacin (40.3%), but showed notable amikacin sensitivity (85.9%).[19] Based on this data, the most effective antibiotics for treating LRTIs in our locations appear to be piperacillin, imipenem, amikacin, vancomycin, and linezolid[26].

This study presented the prevalence of pathogens among patients referred from primary-care hospitals with LRTIs. The pathogens identified encompassed not only *S. pneumoniae* and *H. influenzae* but also *S. aureus, K. pneumoniae*, and *A. baumannii*. Furthermore, this study shed light on antibiotic resistance patterns, which provide crucial insights for selecting appropriate antibiotics within this population. For instance, this study's findings revealed a notable presence of ESBL in 70% of cases and MRSA in 90%. Additionally, within our local area, a high resistance rate of 90% to erythromycin, 40-80% to ciprofloxacin, 30-80% to ceftazidime, and 30-40% to imipenem was observed.

The main limitation of this study was the inclusion of only CAP and AECOPD patients, which could impede the generalizability of the findings to other LRTIs diagnoses. The sample size and the antibiogram count were small. Furthermore, the number of antibiograms was insufficient to analyze risk factors for different types of pathogens, which could not have aided in recommending initial antibiotic treatment based on clinical symptoms.

Conclusion

The spectrum of pathogens responsible for LRTIs referred from primary care hospitals encompasses *S. pneumoniae, H. influenzae, S. aureus, K. pneumoniae,*

and *A. baumannii*. In our specific location, the antibiotics demonstrating the highest sensitivity for effectively treating these LRTIs were piperacillin, imipenem, amikacin, vancomycin, and linezolid.

Contributions

Each of the authors has made a significant intellectual contribution to this work. Additionally, all authors have thoroughly reviewed and endorsed the final version of the manuscript, demonstrating their accountability for all aspects of the research.

Ethics approval and consent to participate

The study was granted approval by the Institutional Review Board to be carried out at Cho Ray Hospital, Pham Ngoc Thach Hospital, Gia Dinh People's Hospital, and Can Tho Central General Hospital. This study was conducted in strict accordance with the principles outlined in the Helsinki Declaration of 1964, and its subsequent amendments.

Informed consent

Written informed consent was obtained from legally authorized representative(s) for anonymized patient information to be published in this article. The manuscript does not contain any identifiable personal data in any form.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Conflict of interest

The authors declare no conflict of interest.

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