Section: Microbiology

Original Research Article

A CROSS-SECTIONAL STUDY ON CLINICAL PRESENTATION WITH CHARACTERISTICS OF ETIOLOGICAL AGENTS, INCLUDING ANTI-MICROBIAL RESISTANCE IN BACTERIAL PNEUMONIA

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Abstract

Background: Acute pneumonia is one of the most common causes of infection related death. The aetiology of pneumonia is difficult to determine by clinical presentation. There is increasing burden of multi drug resistance (MDR) Gram negative pathogens in severe pneumonia cases even in community settings. A limited number of studies are seen with an etiological assessment of pneumonia concerning empiric recommended guidelines. Aim: This study aimed to assess the etiology of pneumonia and the use of accurate anti-microbial therapy. **Material & Methods:** The cross-sectional study was conducted from April 2018 to August 2019 at Govt Tertiary Care Hospital Chennai. The study comprised 210 patients, both Paediatric and Adult patients with clinical signs and symptoms of pneumonia like Major criteria: Cough, Sputum Production, Fever >37.8°C. Minor criteria: Pleuritic chest pain, Dyspnoea, Altered Mental Status, Pulmonary Consolidation by examination, WBC > 11000/mm3 Chest X-ray – Pulmonary infiltration. Patient data such as demographic details, co-morbidities, microbial identification, and drug resistance testing were used to assess pneumonia. Respiratory samples and blood samples for culture were carried out as per standard microbiological techniques. Anti-microbial susceptibility testing was done as per CLSI guidelines. Phenotypic and Genotypic identification of resistant pathogens were also performed. Statistical analysis for the collected data was done using SPSS software. To analyse the significance in the categorical data, Fischer exact test was used. (p-value <0.05 is considered significant.). **Results:** A total of 210 patients with clinical pneumonia were observed, with a male predominance of 135 (64.3%) prevalent in age groups 0-18 years 50 (24%) and 59-68 years 46 (22%). The most common symptoms were fever 200 (95.2%) and cough 190 (90.4%). Diabetes 100 (47.6%) and 60 COPD (28.5%) are prevalent comorbid conditions. Among the study group, 67 patients (32%) reported a positive culture growth of 57 (85%) gram-negative and 10 (15%) gram-positive isolates. Among enterobacterales (33.33%) were found to be ESBL producers. CURB-65 score >2 was reported in 9/34 (26%) of patients with Community-acquired pneumonia and a CPIS score >6 for ventilator-associated pneumonia was seen in 17/24 (71%). Pneumonia recovery was seen in 179 (85.23%) of patients, whereas 31 (14.76%) were observed with mortality. **Conclusion:** Community-acquired pneumonia is one of the morbid infections that increases the risk of mortality. Multi-drug resistance pathogens were prevalent, especially in ventilator-associated pneumonia, and most pneumonia cases required accurate anti-microbial therapy.

INTRODUCTION

An infection of the pulmonary parenchyma causes pneumonia. It is an infection-related inflammation of one or both lungs' parenchyma. In addition to other factors, bacteria, viruses, fungi, and parasites can all cause pneumonia.[1] The new categorization of pneumonia into four subgroups: community-

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acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), Healthcare-associated pneumonia (HCAP) and ventilator-associated pneumonia (VAP).[2] Although many different bacteria can cause pneumonia, in most instances, it is caused by a very small subset. These organisms' virulence and immunogenic traits significantly determine how the host reacts to infection.[3] According to the disease's aetiology, pneumonia has traditionally been divided into "typical" and "atypical" pneumonia. Typical organisms can be demonstrated in Gram staining and cultured in standard bacteriological media, but "atypical" organisms lack these characteristics.[4] Streptococcus pneumoniae, Haemophilus influenza, Staphylococcus aureus, Group A streptococci, Moraxella catarrhalis, anaerobes, and aerobic gram-negative bacteria are all examples of bacterial causes of typical pneumonia. Legionella, Mycoplasma pneumoniae, Chlamydia pneumoniae, and Chlamydia psittaci are the main agents associated with atypical pneumonia.[4] Community-acquired pneumonia (CAP) is most frequently caused by S. pneumoniae, followed by Haemophilusinfluenzae, Mycoplasma pneumoniae, and Chlamydia pneumoniae. The common pathogens causing VAP are Pseudomonas aeruginosa, Acinetobacter species, Klebsiella pneumonia, Enterobacter species and MRSA.[5,6] Among them, Klebsiella pneumoniae, Pseudomonas, and Acinetobacter species are often multi-drug resistant, which is attributed to the production of ESBL, AMPC beta-lactamases, and metallo beta-lactamases.[7] The causes of bacterial pneumonia vary among different populations. Hence, the local microbial flora should be studied, which guides the effective and rational utilisation of anti-microbial agents. The most typical signs of pneumonia include fever, cough, phlegm production, shortness of breath, and chest discomfort.[8] The selection of an adequate empirical anti-microbial therapy is one of the crucial factors for a successful outcome; national and international guidelines provide specific recommendations based on the site of care, pathogen-related risk factors, and multi-drug resistance.[9] Data on the microbiological causes of CAP in countries such as India are scarce and restricted because the data is outdated and constrained by a small sample size. Detection technologies have remained confined to bacterial cultures. While some have indicated Pneumococcus spp. as the primary etiological agent, others have reported Gram-negative bacilli as the most common pathogens.[10] An understanding of the pathogenesis of the disease, evaluation of the relevant data, a careful clinical history and physical examination, recognition of common clinical patterns of infection, and information from the microbiology laboratory all aid in narrowing down the possible etiologic agents of pneumonia. This would aid in the alleviation of morbidity and mortality due to such pathogens.

Resistant genes from bacterial pathogens causing pneumonia were detected by the utility of PCR technology. Resistant genes like TEM AND CTX-M for ESBL producers and NDM in Pseudomonas aeruginosa and OXA-48 in Acinetobacterbaumannii among the gram-negative organisms and mecA gene in MRSA which helps to understand the mechanism of anti-microbial resistance among the pathogens. Also, the increasing burden of MDR gram-negative pathogens in severe pneumonia cases is becoming a major therapeutic challenge, as evidenced in this study, which helps to formulate new antimicrobial stewardship and treatment of pneumonia.

This study aimed to assess the aetiology of pneumonia and the use of accurate anti-microbial therapy. Also, to monitor the incidence and distribution of bacterial pathogens, including antimicrobial resistance and their associated risk factors in the causation of bacterial pneumonia among patients admitted in the medical wards and Intensive Care units.

MATERIALS AND METHODS

This cross-sectional study was conducted at Govt Tertiary Care Hospital Chennai from April 2018 to August 2019. Two hundred ten individuals with clinical pneumonia who met the inclusion criteria were enrolled in the research. 210 (208 samples rounded to 210) were collected using the formula n= 4 × p × q /d2 where p is 75% and q is 25 with d value at 0.06 from the previous literature.[9] For the study, permission from the institutional ethics committee was obtained (Approval No.34122017). Informed consent was also received from the study population.

Inclusion Criteria

Both Paediatric and adult patients with clinical signs and symptoms of pneumonia. Major criteria: Cough, Sputum Production, Fever >37.8°C. Minor criteria: Pleuritic chest pain, Dyspnoea, Altered Mental Status, Pulmonary Consolidation by examination, WBC > 11000/mm3 Chest X-ray – Pulmonary infiltration.

Exclusion Criteria

Patients with congestive heart failure and chronic lung diseases such as Tuberculosis, Emphysema, Atypical pneumonia, and carcinoma lung.

The medical records including name, age, sex, ward, date of admission, chief complaints, past medical history, level of consciousness, risk factors such as hypertension, diabetes, chronic obstructive pulmonary disease and chronic kidney disease were recorded.

Sample collection transport and processing.[11,12,13]

Clinical samples from the patients, including sputum, endotracheal aspirates, Bronchoalveolar lavages, gastric aspirates, and blood, were taken under stringent aseptic conditions and transported immediately to the microbiology laboratory.

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Respiratory (Sputum, Endotracheal aspirate, Broncho Alveolar Lavage, and Gastric aspirate) samples were mechanically homogenized by vortexing for one minute before being examined microscopically using conventional laboratory procedures. Direct examination of gram-stained preparations was performed and studied for the presence of squamous epithelial cells, polymorphonuclear cells, bacteria (Gram-positive and Gram-negative), and their morphology.

Under aseptic care, samples such as sputum, gastric aspirate, endotracheal aspirate and bronchoalveolar lavage were inoculated using conventional methods on standard bacteriological media. The purulent portion of the sputum was washed in 5 ml of sterile physiological saline, and the washed sputum was inoculated into plates of blood agar, chocolate agar and MacConkey agar. Chocolate and blood agar plates were kept in 5- 10% CO2 and incubated at 37°C.

Identifying and classifying pathogens and colonizers were done quantitatively on endotracheal aspirate and bronchoalveolar lavage (ETA/BAL) specimens. Serial dilutions of the specimens were performed with sterile normal saline as 1/10, 1/100, 1/1000, and 0.01 ml of these dilutions were used to inoculate 5% sheep blood agar, Mac Conkey agar and chocolate agar. Colony counts were performed and expressed as the number of colony-forming units per ml (CFU/ml) after incubation at 37°C for 24 to 48 hours.[14]

Colony forming units per millilitre (CFU/ml), representing the number of bacteria in the initial sample, are calculated as follows: number of colonies x dilution factor x inoculation factor. Bacterial growth with colony counts of 105 CFU/ml (Endotracheal aspirate) and 104 CFU/ml (BAL) was defined as Pathogens. Any organisms that grew below the threshold were classified as contaminants or colonizers.[6,14] The plates that showed threshold growth were studied by colony morphology and Gram reaction and identified using standard biochemical reactions. After initial characterization of the isolates by colony morphology, Gram stain, species identification and susceptibility testing were done. In blood culture bottles, microbial growth was detected by macroscopic examination of bottles and blind and terminal subcultures were done after 7 days of incubation.[15]

Anti-microbial susceptibility testing.[12]

Anti-microbial susceptibility testing was done using Kirby Bauer's disc diffusion method on Mueller Hinton agar based on CLSI guidelines 2019. The liquid culture of the test isolate adjusted to 0.5 McFarland turbidity was inoculated by streaking the swab three times over the entire agar surface of the Mueller Hinton agar plate, and antibiotic discs were placed on the surface of the agar based on the growth of gram-positive or gram-negative organisms. The plates were incubated at 37°C overnight. The zone of inhibition was measured from the center of the disc to the edge of the area with zero growth on the agar surface and interpreted as per CLSI guidelines 2019.

Interpretation of clinical and microbiological criteria.[4, 16-20]

Ventilator-associated pneumonia and community-acquired pneumonia were catagorised based on both the clinical and microbiological characteristics. Among the community acquired pneumonia (CAP) patients, CURB 65 score was evaluated as C-CONFUSION based upon a specific mental test or new disorientation to person, place or time, U-UREA > 7mmol/L (20mg/Dl), R-respiratory rate > 30 breaths/min, Blood pressure systolic <90mmHg or diastolic <60 mmHg, and age >65 years.

Clinical pulmonary infection score (CPIS) calculated based on Temperature<36.5 *C and pao2/FIO2mm/Hg>240, chest X-Ray any infiltrate or consolidation, Quantitative pathogenic bacterial culture were calculated from patients (CPIS) scores of >6 were diagnosed with Ventilator-associated pneumonia.

Modified clinical pulmonary infection score >6 for Ventilator-associated pneumonia and CURB-65 >2 score in Community-acquired pneumonia. The quality of the sputum sample was evaluated as per the Bartlett grading system. For evaluating sputum samples for the relative number of squamous epithelial cells and segmented neutrophils, a Direct gram stain of the sputum sample was done using scoring and grading; negative numbers were assigned to a smear when squamous epithelial cells were observed indicating contamination with oropharyngeal secretions(saliva). Positive numbers were assigned for the number of segmented neutrophils indicates the presence of active inflammation.

Bartlett's grading system for assessing the quality of sputum samples,[21]

No. of neutrophils per 10x low power field	Grade
<10	0
10-25	+1
>25	+2
Presence of mucus	+1
No of epithelial cells per 10x low power field	**Grade**
10-25	-1
>25	-2

Positive endotracheal aspirate culture findings revealed 105 CFU/ml and positive Gram stain (more than 10 polymorphonuclear cells/Low power field and 1 bacteria/Oil immersion field) for Ventilator-associated pneumonia.

Detection of anti-microbial resistance causing enzymes among bacterial isolates,[12]

Phenotypic assessment of extended-spectrum beta-lactamase (ESBL) by ESBL screening and phenotypic confirmation by combination disc method, Metallobeta-lactamase enzyme was detected by Imipenem EDTA combined disc test among the gram-negative bacterial pathogens and methicillin resistance testing in gram-positive bacteria was done by cefoxitin (30µg) disc diffusion method.

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Molecular characterization of resistant bacterial isolates

PureFast® Bacterial DNA Extraction Kit from HELINI Biomolecules, Chennai. Tamilnadu.[22]

2X master mix: It contains 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl2, 1µl of 10mM dNTPs mix and RedDye PCR additives). The following primers were used to detect the resistant genes from resistant pathogens. blaNDM1 gene Primer mix - 5µl/reaction PCR Product: 214bp. blaCTX-M gene Primer mix - 5µl/reaction PCR Product: 295bp. MecA gene Primer mix - 5µl/reaction PCR Product: 220bp. blaTEM gene Primer mix -5µl/reaction

Extraction of DNA: 1ml of overnight culture was centrifuged at 6000rpm for 5 min, and Pellet was suspended in 0.2ml Phosphate buffer saline after adding the 180µl of Lysozyme digestion buffer and 20µl of Lysozyme [10mg/ml] was Incubated at 37C for 15min. Then. Binding buffer, internal control template and Proteinase K were added and mixed, Incubated at 56ºC for 15min. After adding (300 µl) ethanol, the entire sample was put into the PureFast® spin column. Centrifuged for 1 min. The flow-through was discarded, and the column was placed back into the same collection tube. Centrifugation was done twice for 30-60 seconds after adding 500µl Wash buffer-1 & 2. The flow-through was discarded and centrifuged for an additional 1 min. Then, 100µl of Elution Buffer was added to the mixture, transferred the PureFast® spin column into a fresh 1.5 ml micro-centrifuge tube, incubated for 1 min at room temperature, and centrifuged for 2 min, and the purified DNA was stored at -20°C.

Amplification of DNA: PCR Reactions are set up as follows;

Components Quantity

RedDye PCR Master mix - 10µl
Primer Mix - 5µl
Purified Bacterial DNA - 10µl
Total volume - 25µl

After a brief spinning mixture was Placed into the PCR machine and programmed as follows;

Initial Denaturation: 95ºC for 5 min
Denaturation: 94ºC for 30sec
Annealing: 58ºC for 30sec 35 cycles
Extension: 72ºC for 30sec
Final extension: 72º C for 5 min

Agarose gel electrophoresis: 2% agarose gel platform was placed into a tank and set undisturbed until the agarose solidified. Then PCR Samples were loaded after being mixed with gel loading dye and 10µl of 100bp DNA Ladder. [100bp, 200bp, 300bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp,1000bp and 1500bp]. Electrophoresis was run at 50V till the dye reached three fourth distance of the gel. Then the gel was viewed in UV Transilluminator and observed the bands pattern detected the presence of DNA.

Statistical Analysis

All results were tabulated into MS Excel format. Analysis was done using mean and proportions or percentage. Fisher exact test was used in all tables and the p value of <0.05 was considered significant with the help of the SPSS software version 2.2.

RESULTS

The 210 patients in the study group, including adults and children, who had been hospitalized with symptoms and signs of clinical pneumonia were analyzed as follows. Out of 210 patients, 135 (64.3%) were males, and 75 (35.7%) were females. Among them, age groups 0-18 years 50 (24%) and 59-68 years 46 (22%) were predominantly affected by pneumonia. [Table 1]

Predominant symptoms among the patients with clinical pneumonia were fever 200 (95.2%) and cough with expectoration 190 (90.4%). [Table 2]

Out of 210 patients, co-morbidities and risk factors associated with bacterial pneumonia showed Diabetes mellitus 100 (47.6%), followed by COPD 60 (28.5%) and elderly patients >65 (28.5%) among the cases. [Table 3]

Clinical samples obtained from patients with clinical pneumonia 110 (52.4%) were sputum followed by bronchoalveolar lavage 44 (21%), endotracheal aspirate and tracheal aspirate 24 (11.4%) each, and gastric aspirate 8 (3.8%). [Table 4]

In the present study, the association between direct microscopy vs quantitative culture of Endotracheal aspirate [Table 5] and BAL [Table 6] showed significant growth 10^5 and 10^4, respectively.

In this study, the correlation between direct microscopy vs culture of sputum showed significant growth.

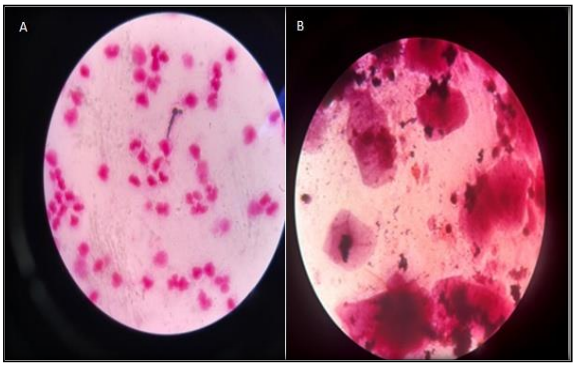

Figure 1: Images of direct gram stain of sputum (A) more than 25 pus cells (B) more than 10 epithelial cells (Magnification 100X) Showing the Quality of Sputum Samples
A. Good quality sample B. Poor quality sample

Out of 210 samples in this study, 67(32%) were culture-positive. Among them, 57 (85%) were gram-negative, and 10 (15%) were gram-positive bacterial pathogens. [Table 7]

Among 27 Enterobacterales, 9 (33.33%) were found to be ESBL producers.

Figures 2A and 2B

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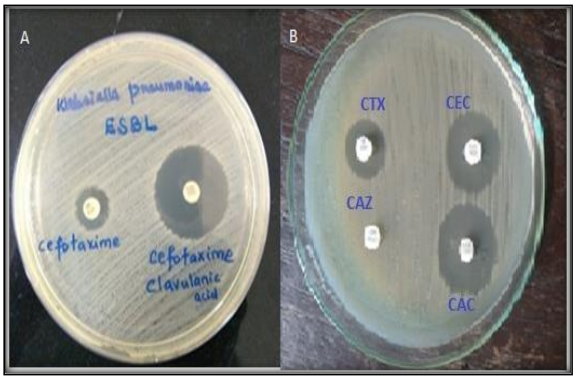

Figure 2: Production of extended-spectrum beta-lactamases - Klebsiella pneumonia (A) screening test (B) Confirmatory test

Among the clinical samples, culture positivity in sputum 32 (48%), Endotracheal and Tracheal aspirate 24 (36%), and Bronchoalveolar lavage 11 (16.4%). The predominant pathogen in Sputum culture was Klebsiella pneumonia 10 (31.2%), Klebsiella pneumoniae (ESBL) 7 (22%), Acinetobacter baumannii 5 (16%) and Streptococcus pneumonia 2 (6.3%) and Staphylococcus aureus (MRSA) and Staphylococcus aureus (MSSA) 2 (6.3%) each [Table 8].

Bronchoalveolar lavage of patients with clinical pneumonia was found to have Acinetobacter baumannii and Pseudomonas aeruginosa each 3 (27.3%), followed by Klebsiella pneumonia 2(18.2%), Klebsiella pneumoniae (ESBL) 1 (9.1%) and Staphylococcus aureus (MSSA) 2 (18.2%). [Table 9]

Tracheal aspirate and Endotracheal aspirate cultures from patients with pneumonia contributed to Acinetobacter baumannii 12 (50%), followed by Klebsiella pneumonia 5 (21%) and Pseudomonas aeruginosa 5(21%) and Staphylococcus aureus (MRSA) 2 (8.3%). [Table 10]

Out of 210 cases, blood culture was positive in 9 patients. [Table 11]

In this study, three patients with clinical pneumonia had shown growth of two bacterial isolates from Endotracheal and Tracheal aspirates of VAP cases.

In the present study, the gram-negative isolates were highly sensitive to the antibiotics Piperacillin-tazobactam, Carbapenems and Amikacin and gram-positive cocci like Streptococcus pneumonia were found to be susceptible to Amoxyclav and Penicillin.

Carbapenem resistance observed among the non-fermenters such as Pseudomonas aeruginosa and Acinetobacter baumannii, was found to be 4/10(40%) and 6/20(30%) from VAP cases respectively. But enterobacterales Carbapenem resistance was seen in 2/9(22.2%) of cases. [Table 13]

Molecular characterization of resistant isolates showed the presence of bla TEM and bla CTX-M genes (ESBL producers), bla OXA-48 in Acinetobacter baumannii, and bla NDM in Pseudomonas aeruginosa and mec-A gene for staphylococcus aureus. Gel documentation images showing amplified gene products using 100bp DNA ladder in Pseudomonas aeruginosa, Acinetobacter baumanii and Staphylococcus aureus (MRSA).

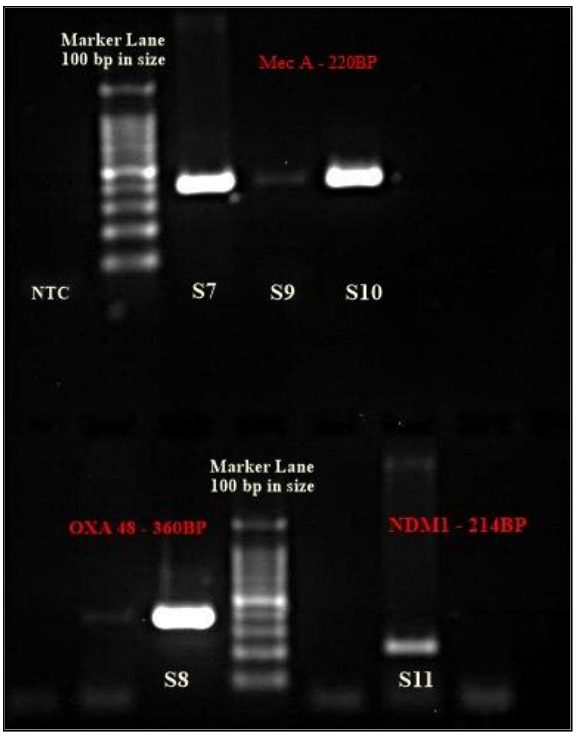

Figure 3: T0P; mec A Gene in (MRSA), BOTTOM; Detection of bla OXA48 in Acinetobacter baumannii and bla NDM in Pseudomonas aeruginosa

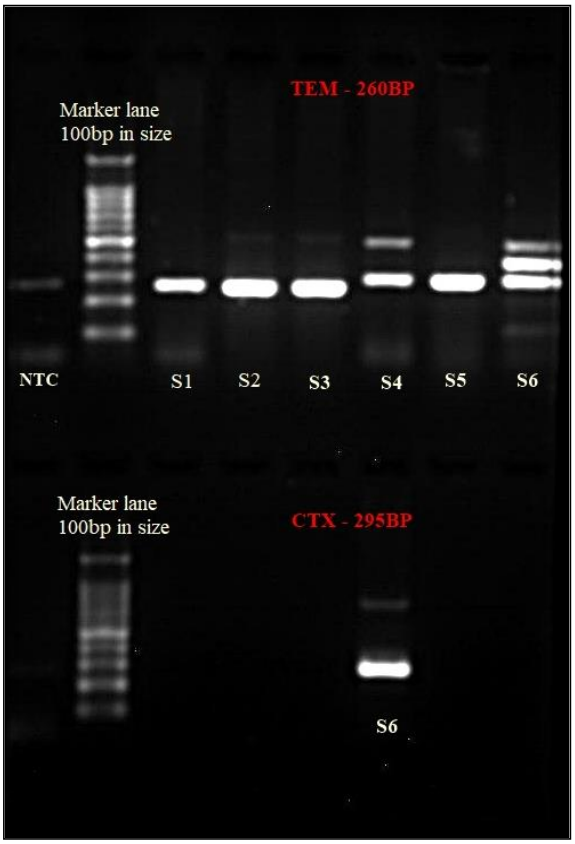

Figure 4: TOP Detection of bla TEM, BOTTOM; bla CTX-M Klebsiella pneumonia (ESBL)

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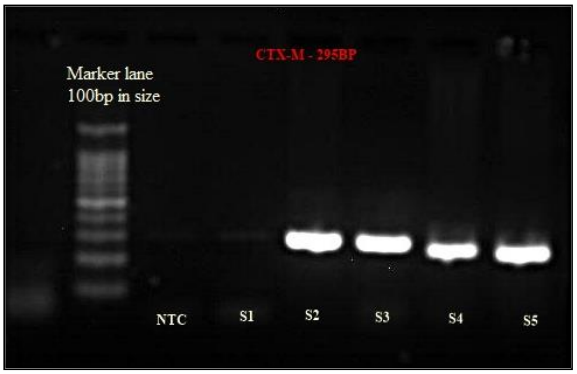

In the present study, among clinical pneumonia, ventilator-associated pneumonia was 24/48 (50%) followed by hospital-acquired pneumonia 4/10 (40%) and community-acquired pneumonia 34/152 (22.3%) [Figure 4].

Among 210 patients with Clinical pneumonia, 179 (85.23%) recovered and discharged 31(14.76%) were expired [Table/Fig 15].

[Table 16] Out of 34 cases community-acquired pneumonia 9 (26%) number of patients of had CURB-65 score >2 is and were admitted and treated for the same. Out of 24 cases of Ventilator-associated pneumonia 17 (71%) of cases were found to be (CPIS) scores of >6.

Table 1: Age distribution of patients with clinical pneumonia (n=210)

Age group	Number (%)
Up to 18 years	50 (24)
19 - 28 years	17(8.1)
29 - 38 years	14 (6.7)
39 - 48 years	28(13.3)
49 - 58 years	31(14.8)
59 - 68 years	46(22)
Above 68 years	24(11.4)
Total	210(100)

Table 2: Clinical profile of patients with suspected pneumonia

Clinical symptoms	Number (%)
Fever	200(95.2)
Cough with expectoration	190(90.4)
Pleuritic chest pain	52 (24.7)
Breathlessness	50(23.8)
Altered sensorium	40(19.04)
Haemoptysis	20(9.5)
Gastro intestinal symptoms	10(4.7)

Table 3: Co-morbidities and risk factors associated with bacterial pneumonia

Co-morbidities and risk factors	No of patients (%)
Diabetes	100(47.6)
COPD	60(28.5)
Elderly >65	60(28.5)
Smoking	56(26.6)
Alcoholic	50(23.8)
Hypertension	50(23.8)
Prolonged stay in hospital	20(9.5)
Renal disease	10(4.7)

Table 4: Sample distribution from patients with clinical pneumonia

Sample distribution	Number (%)
Sputum	110(52.4)
Broncho alveolar lavage	44(21)
Endotracheal aspirate	24(11.4)
Tracheal aspirate	24(11.4)
Gastric aspirate	8(3.8)

Table 5: Association between direct microscopy vs quantitative culture of endotracheal aspirate and tracheal aspirate

Direct gram stain	Quantitative culture			P Value
	NG	10^5	Total	
>10 cells	11	14	25	0.037#
1-10 cells	0	2	2	
0 cells	17	4	21	
Total	28	20	48	

[# Fisher exact test is used because are >20% of expected cell counts are <5]

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Table 6: Association between direct microscopy vs quantitative culture in bronchoalveolar lavage

Direct gram stain	Quantitative culture			P Value
Direct gram	NG	104	Total	
>10 cells	7	8	15	#0.018 Fisher exact test
1-10 cells	2	2	4	
0 cells	23	2	25	
Total	32	12	44	

[#Fisher exact used since >20% of the expected cell counts are <5]

Table 7: Culture positivity among the patients with clinical pneumonia (n=67)

Group	Isolates	Number (%)
GPC (10 isolates) 15%	Staphylococcus aureus MSSA	4(6%)
	Staphylococcus aureus MRSA	4(6%)
	Streptococcus pneumoniae	2(3%)
GNB (57 isolates) 85%	Klebsiella pneumoniae	17(25.3%)
	Klebsiella pneumoniae (ESBL)	8(12%)
	Acinetobacter baumannii	20(30%)
	Pseudomonas aeruginosa	10(14.9)
	Klebsiella oxytoca	1(1.4)
	Escherichia coli (ESBL)	1(1.4)
Total		67 (32)

Table 8: Bacterial isolates from sputum cultures of patients with clinical pneumonia (n=32)

Bacterial isolates	Number (%)
Klebsiella pneumoniae	10(31.2%)
Klebsiella pneumoniae (ESBL)	7(22%)
Acinetobacter baumannii	5(16%)
Klebsiella oxytoca	1(3.1%)
Pseudomonas aeruginosa	2(6.3%)
Staphylococcus aureus (MRSA)	2(6.3%)
Staphylococcus aureus (MSSA)	2(.6.3%)
Streptococcus pneumoniae	2(6.3%)
Escherichia coli (ESBL)	1(3.1%)
Total	32(100%)

Table 9: Bacterial isolates in bronchoalveolar lavage from patients with clinical pneumonia (n=11)

Bacterial isolates	Number (%)
Acinetobacter baumannii 10^4	3(27.3)
Pseudomonas aeruginosa 10^4	3(27.3)
Klebsiella pneumonia 10^4	2(18.2)
Klebsiella pneumonia (ESBL) 10^4	1(9.1)
Staphylococcus aureus (MSSA) 10^5)	2(18.2)
Total	11(100.0)

Table 10: Bacterial isolates from endotracheal and tracheal aspirates from patients with clinical pneumonia (n=24)

Bacterial isolates	Number (%)
Acinetobacter baumannii 10^5	12(50)
Klebsiella pneumoniae 10^5	5(21)
Pseudomonas aeruginosa 10^5	5(21)
Staphylococcus aureus (MRSA) 10^5	2(8.3)
Total	24(100)

Table 11: Bacterial isolates from blood cultures of patients with pneumonia (n=9)

Blood culture	Number (%)
Acinetobacter baumannii	3(33.3)
Pseudomonas aeruginosa	2(22.2)
Staphylococcus aureus (MRSA)	2(22.2)
Staphylococcus aureus (MSSA)	1(11.1)
Klebsiella pneumoniae	1(11.1)
Total	9(100)

Table 12: Sample-wise distribution of polymicrobial isolates from patients with clinical pneumonia

Bacterial isolates	Tracheal aspirate	Endo tracheal	Bal	Sputum	Gastric aspirate	Total
1.Klebsiella pneumoniae 10 ^ 5 2.Pseudomonas aeruginosa 10 ^5	1	0	0	0	0	1
1.Staphylococcus aureus (MRSA)10 ^ 5 2.acinetobacter baumanii10 ^ 5	1	0	0	0	0	1
1. Acinetobacterbaumanii 10^5 2. Staphylococcus aureus 10 ^5 (MRSA)	0	1	0	0	0	1

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Table 13: Carbapenem Resistance among the pathogens

Bacterial isolates	Carbapenem resistance observed (n%)
Pseudomonas aeruginosa (10)	4(40%)
Acinetobacterbaumannii (20)	6(30%)
Enterobacterales (9)	2(22.2%)

Table 14: Vancomycin susceptibility among MRSA isolates

Vancomycin MIC(µg/ml)	Susceptible≤ 2	Intermediate4-8	Resistant≥16
Staphylococcus aureus (MRSA)[4]	4	0	0

Table 15: Categorisation of patients with clinical pneumonia and outcome

		Positive patients	Negative Patients	Total no of cases
Pneumonia category	Community-acquired pneumonia	34 (22.3%)	118(77.6%)	152
	Ventilator-acquired pneumonia	24 (50%)	24(50%)	48
	Hospital-acquired pneumonia	4(40%)	6(60%)	10
Outcome	Recovered	179(85.23%)		
	Expired	31(14.76%)		

Table 16: Scoring for CAP and VAP

Pneumonia category	Score	Number (%)
Community acquired pneumonia	CURB score>2	9/34 (26%)
Ventilator associated pneumonia	CPIS score >6	17/24(71%)

Table 17: CPIS score vs ventilator associated pneumonia

CPIS score	No. of patients
<6	7
>6	17

DISCUSSION

Community-acquired pneumonia (CAP) is a leading cause of morbidity and mortality worldwide. The clinical presentation of CAP varies, ranging from mild pneumonia characterized by fever and productive cough to severe pneumonia characterized by respiratory distress and sepsis. Because of the wide spectrum of associated clinical features, CAP is a part of the differential diagnosis of nearly all respiratory illnesses.[23] This study was done to know the clinical presentations in correlation with characteristics of etiological agents, including anti-microbial resistance. In the present study on bacterial pneumonia, there was a male preponderance (64.3%) than female (35.7%), and patients in the age group 0-18 years (24%) and 59-68 years (22%) were more affected. Prasad et al. also reported that males were more affected with bacterial pneumonia, and both extremes of age group were affected.[24]

In the present study, clinical presentations among the patients, the predominant symptoms were fever (95.2%) and cough with expectoration (90.4%). Similar studies also observed fever and cough as the more common symptoms in patients with bacterial pneumonia, which agree with our study.[25,26] The present study most common predisposing factor for pneumonia was diabetes mellitus (47.6%), COPD, and elderly patients (28.5%). In this study, the Quantification of Endotracheal aspirate and BAL is well correlated with more pus cells, and culture showed correspondingly 10^5 and 10^4 CFU/ml growth. The larger variation in the observations of Gram staining of Endotracheal aspirate and sputum leads to conclude that Gram stain of these specimens was of very limited value in providing information useful for diagnosing and managing patients known or suspected to have lower respiratory tract infections. Cultures of expectorated sputum and tracheal aspirates are more useful.[27]

In the present study, the quality of the sputum is assessed by the Bartlett scoring system. Sputum samples showed >25 polymorphonuclear cells, and bacterial growth was significant in culture, which is well matched with the findings of Cilloniz et al. study.[28] In the present study, among the culture-positive patients, Klebsiella pneumonia (31.2%) was the most common pathogen in sputum samples. Among the Gram-positive bacterial isolates Streptococcus pneumoniae and Staphylococcus aureus (MRSA&MSSA),6.3% each in sputum samples were identified. In this study, three patients had shown growth of two bacterial isolates. In recent years, there has been an increase in recognition of the gram-negative bacterial etiology of CAP due to several factors, including more effective microbiological research, a trend toward a worsening of illness severity in patients admitted to the ICU, and an increase in life expectancy as older patients experience CAP more frequently colonized by GNB.[29] In the present study, Acinetobacterbaumannii (50%) was the most common pathogen, then Pseudomonas aeruginosa and Klebsiella pneumonia 10^5CFU/ml each 21% in Endotracheal aspirate and Tracheal aspirate from VAP. Several studies have reported that aerobic GNB causes more than 60% of VAP.[30,31]

Nine individuals (4.3%) of the 210 cases in this research had positive blood cultures. Blood cultures are relatively insensitive to diagnose pneumonia. The rapid availability of cytological data, including

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inflammatory cells and Gram stains, may be useful in initial therapeutic decisions in patients with suspected VAP.[32] In the present study, the gram-negative isolates were highly sensitive to the antibiotics piperacillin-tazobactam, carbapenems and amikacin. Also, the present study showed Streptococcus pneumoniae from community-acquired pneumonia cases were susceptible to Penicillin and Amoxyclav. Penicillin resistance among S. pneumoniae is a global problem. Earlier, three-year surveillance for penicillin resistance from Vellore revealed 4.6% of intermediate resistance to penicillin,[33] whereas a North Indian study reported 15.2% (26/170) intermediate resistance and 2.3% (4/170) penicillin resistance.[34] Recent reports show that macrolide resistance in S. pneumoniae is geographically variable, ranging from 30 to 50% globally (Sader et al., 2018; Wang et al., 2019; Sharew et al., 2021). Our study found that Methicillin-resistant Staphylococcus aureus from endotracheal and tracheal aspirates are susceptible to vancomycin. Among enterobacterales (33.33%) were found to be ESBL producers. Patients with ventilator-associated pneumonia were found to have bacterial pathogens such as Acinetobacter baumannii and Pseudomonas aeruginosa that were more resistant to numerous anti-microbial medications.[35,36]

In the present study, molecular characterization of resistant pathogens using Polymerase chain reaction, bla-CTX-M, and blaTEM genes was detected in Klebsiella pneumoniae (ESBL) producers and among the non-fermenting gram-negative bacilli, bla (Oxa-48) gene was seen in MDR Acinetobacterbaumannii and (bla NDM) in Pseudomonas aeruginosa. These findings were similar to the study done by Xu et al., where K. pneumoniae isolates from mechanically ventilated patients, the blaCTX-M-15 gene was co-transferred to the recipient strain with bla TEM.[36]

In the present study, out of 24 patients of VAP 17 (71%) patients had CPIS scores of > 6. Accurate tracheal aspirate gram staining can guide early empiric antibiotic therapy and may boost the diagnostic value of the CPIS, according to the American Thoracic Society's recommended practices.[37]

The extended CURB -65 is a more accurate and user-friendly scoring system for assessing CAP. It needs hospitalization if the score is more than 2. If it is, less than 2 can be treated as outpatients. In this study, 26% of patients were hospitalized and treated for community-acquired pneumonia because they had a CURB-65 score of >2, similar to the findings of Liu et al. [38]. CURB-65 was simpler and more efficient among all scoring systems. In this study outcome of patients with bacterial pneumonia, 85.23% recovered and were discharged in good condition and 14.76% mortality. Similarly, in a study done by Osman et al. in-hospital mortality during their study period was 17.6%, and it was as high as 45% for patients admitted to the ICU.[39]

Further to this, the mortality rate due to CAP in hospital-based studies has varied where the British Thoracic Society multicentre study reported a mortality of 5.7%,[40] and 4% was reported by Ortqvist et al,[41] and Pachon et al,[42] with 20.8%. The mortality rates in patients vary based on the hospitalization history. Costa et al,[43] in a hospital-based study, reported a mortality rate of 11.7% in hospitalized patients with bacteremia and P. aeruginosa as an independent risk factor for increased mortality. Similar data was also represented by an Asian study by Kang et al., where GNB infections were associated with higher mortality rates.[44]

The higher incidence of multi-drug resistance results in poor therapeutic outcomes, especially in VAP patients resulting in high mortality and loss of life. Hence, it is essential to acquire and follow the anti-microbial guidelines for empirical treatment and use bacterial culture tests to identify the causative organism of pneumonia.

Limitations of the study

The single centre study, small sample size and difficulty to identify the fastidious pathogens causing pneumonia due to prior antimicrobial treatment are some of the limitations

CONCLUSION

In both nosocomial and community-acquired illnesses, bacterial pneumonia poses a serious concern. Pathogens with rising degrees of multi-drug resistance are especially prevalent in ventilator-associated pneumonia. This investigation helped to identify the causal infectious agents, their anti-microbial susceptibility patterns and clinical data. In communal settings, ESBL producers become the common resistant strains. In ventilator patients, Methicillin-resistant Staphylococcus aureus and Acinetobacterbaumannii were the most frequently identified pathogens. Anti-microbial resistance is a significant potential issue. Hence, it's important to identify resistant bacteria through effective surveillance. Tracking antimicrobial-resistant bacteria in nosocomial and community settings is easier through the molecular characterization of resistant infections, and effective antibiotic policies and preventive measures must be developed to stop the spread of these antimicrobial-resistant bacteria in healthcare facilities and communal settings.

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REFERENCES

1. Sattar SBA, Sharma S. Bacterial Pneumonia. StatPearls Publishing; 2022.
2. Mandell LA, Wunderink RG. Pneumonia. In: Kasper DL, Fauci AS, eds. Harrison's Infectious Diseases. 3rd ed. New York: McGraw-Hill Education; 2015. p. 217.
3. Mosier DA. Bacterial pneumonia. Vet Clin North Am Food Anim Pract. 1997;13(3):483-493.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

4. Pahal P, Rajasurya V, Sharma S. Typical Bacterial Pneumonia. [Updated 2023 Jul 31]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534295/
5. Harrison's infectious diseases 3rd edition Dennis. Kasper Anthonys. Fauci 21st chapter pneumonia. Pg 217.
6. Joseph NM, Sistla S, Dutta TK, Badhe AS, Parija SC. Ventilator-associated pneumonia: a review. Eur J Intern Med 2010; 21:360–8.
7. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005; 171:388–416.
8. Mandell, Douglas and Bennett's Acute Pneumonia JenniferS, Daily and R. In John E. Bennett, Raphael Dolin, Martin J. Blaser, Principles and Practice of Infectious Diseases, Ninth edition pp 885-894.
9. Bansal S, Kashyap S, Pal LS, Goel A. Clinical and bacteriological profile of community-acquired pneumonia in Shimla, Himachal Pradesh. Indian J Chest Dis Allied Sci. 2004; 46:17–22.
10. Reyaz A, Bashir A, Jan S, Shah PA. Microbial etiology in hospitalized North Indian adults with community-acquired pneumonia 2018. Mar-Apr 2018; 35:108–15.
11. Winn, W, Allen, S, Janda W, Koneman E, Procop G, Schreckenberger P. Woods G. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Edition, Lippincott Williams and Wilkins 2006.
12. Performance standards for Anti-Microbial Susceptibility Testing; Twenty Seventh Informational supplement. CLSI document M100-S27.
13. Rajasekhar T, Anuradha K, Suhasini T, Lakshmi V. The role of quantitative cultures of non-bronchoscopic samples in ventilator-associated pneumonia. Indian J Med Microbiol 2006; 24:107–13.
14. Kirtland SH, Corley DE, Winterbauer RH, Springmeyer SC, Casey KR, Hampson NB, et al. The diagnosis of ventilator-associated pneumonia: a comparison of histologic, microbiologic, and clinical criteria. Chest 1997; 112:445–57.
15. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia. Clin Microbiol Rev 1997; 10:444–65.
16. Napolitano LM. Use of severity scoring and stratification factors in clinical trials of hospital-acquired and ventilator-associated pneumonia. Clin Infect Dis 2010;51 Suppl 1: S67-80.
17. Koenig SM, Truwit JD. Ventilator-associated pneumonia: diagnosis, treatment, and prevention. ClinMicrobiol Rev. 2006;19(4):637–57.
18. Porzecanski I, Bowton DL. Diagnosis and treatment of ventilator-associated pneumonia. Chest. 2006;130(2):597–604.
19. Wu CL, Yang DI, Wang NY, Kuo HT, Chen PZ. Quantitative culture of endotracheal aspirates in the diagnosis of ventilator-associated pneumonia in patients with treatment failure. Chest. 2002;122(2):662–8
20. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. Am Rev Respir Dis. 1991 May;143(5 Pt 1):1121-9.
21. Washington WJ, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods G, et al. Koneman's Color Atlas and Text Book of Diagnostic Microbiology. 6th ed. Baltimore: Lippincott Williams & Wilkins; 2006.
22. Tile P.M., Nucleic acid based Analytical methods for microbial identification and characterisation. Bailey & Scott's Diagnostic Microbiology. 13th edition (2014):112-12
23. Julio A Ramirez, MD, FACP Overview of community-acquired pneumonia in adults up to date Apr 13, 2023.
24. Prasad P, Bhat S. Clinicomicrobiological study of community-acquired pneumonia. Lung India. 2017; 34:491-2.
25. Metlay JP, Fine MJ, Schulz R, Marrie TJ, Coley CM, Kapoor WN, et al. Measuring symptomatic and functional recovery in patients with community-acquired pneumonia. J Gen Intern Med 1997; 12:423–30.
26. Claudius I, Baraff LJ. Pediatric emergencies associated with fever. Emerg Med Clin North Am 2010; 28:67–84, vii–viii.
27. Nagendra S, Bourbeau P, Brecher S, Dunne M, LaRocco M, Doern G. Sampling variability in the microbiological evaluation of expectorated sputa and endotracheal aspirates. J Clin Microbiol 2001; 39:2344–7.
28. Cilloniz C, Martin-Loeches I, Garcia-Vidal C, San Jose A, Torres A. Microbial etiology of pneumonia: Epidemiology, diagnosis and resistance patterns. Int J MolSci 2016; 17:2120.
29. Shah BA, Singh G, Naik MA, Dhobi GN. Bacteriological and clinical profile of community-acquired pneumonia in hospitalized patients. Lung India 2010; 27:54–7.
30. Chastre J, Fagon J-Y. Ventilator-associated pneumonia. Am J RespirCrit Care Med 2002; 165:867–903.
31. Cojuc-Konigsberg G, Moscona-Nissan A, Guijosa A, Mireles Dávalos CD, Martínez ME, Mújica Sánchez MA, et al. Diagnostic accuracy of the BioFire® FilmArray® pneumonia panel in COVID-19 patients with ventilator-associated pneumonia. BMC Infectious Diseases. 2023 Aug 9;23(1):524.
32. Rea-Neto A, Youssef NCM, Tuche F, Brunkhorst F, Ranieri VM, Reinhart K, et al. Diagnosis of ventilator-associated pneumonia: a systematic review of the literature. Crit Care 2008;12: R56.
33. Lalitha MK, Pai R, Manoharan A, Appelabaum PR, Singh NP, Kaur M, et al. Prospective multicenter hospital surveillance of Streptococcus pneumoniae disease in India. Invasive Bacterial Infection Surveillance (IBIS) Group. International Clinical Epidemiology Network (INCLEN) Lancet 1999; 353:256–9.
34. Goyal R, Singh NP, Kaur M, Talwar V. Antimicrobial resistance in invasive and colonising streptococcus pneumoniae in north India. Indian J Med Microbiol 2007; 25:256–9. https://doi.org/10.1016/s0255-0857(21)02117-4.
35. Chittawatanarat K, Jaipakdee W, Chotirosniramit N, Chandacham K, Jirapongcharoenlap T. Microbiology, resistance patterns, and risk factors of mortality in ventilator-associated bacterial pneumonia in a Northern Thai tertiary-care university based general surgical intensive care unit. Infect Drug Resist. 2014 Aug 16; 7:203-10.
36. Xu H, Huo C, Sun Y, Zhou Y, Xiong Y, Zhao Z, Zhou Q, Sha L, Zhang B, Chen Y. Emergence and molecular characterization of multidrug-resistant Klebsiella pneumoniae isolates harboring blaCTX-M-15 extended-spectrum β-lactamases causing ventilator-associated pneumonia in China. Infect Drug Resist. 2018 Dec 20; 12:33-43.
37. Dennesen PJW, van der VEN AJAM, Kessels AGH, Ramsay G, Bonten MJM. Resolution of infectious parameters after anti-microbial therapy in patients with ventilator-associated pneumonia. Am J Respir Crit Care Med 2001; 163:1371–5.
38. Liu J-L, Xu F, Zhou H, Wu X-J, Shi L-X, Lu R-Q, et al. Expanded CURB-65: a new score system predicts severity of community-acquired pneumonia with superior efficiency. Sci Rep 2016; 6:22911.
39. Osman M, Manosuthi W, Kaewkungwal J, Silachamroon U, Mansanguan C, Kamolratanakul S, et al. Etiology, clinical course, and outcomes of pneumonia in the elderly: A retrospective and prospective cohort study in Thailand. Am J Trop Med Hyg 2021; 104:2009–16.
40. The British thoracic society and the public health laboratory serviceCommunity-acquired pneumonia in adults in British hospitals in 1982-1983: A survey of aetiology, mortality, prognostic factors and outcome. QJM 1987; 62:195–220.
41. Ortqvist A, Hedlund J, Grillner L, Jalonen E, Kallings I, Leinonen M, et al. Aetiology, outcome and prognostic factors in community-acquired pneumonia requiring hospitalization. Eur Respir J 1990; 3:1105–13.
42. Pachon J, Prados MD, Capote F, Cuello JA, Garnacho J, Verano A. Severe community-acquired pneumonia: Etiology, prognosis, and treatment. Am Rev Respir Dis 1990; 142:369–73.
43. Costa MI, Cipriano A, Santos FV, Valdoleiros SR, Furtado I, Machado A, et al. Clinical profile and microbiological aetiology diagnosis in adult patients hospitalized with community-acquired pneumonia. Pulmonology 2022; 28:358–67.
44. Kang C-I, Song J-H, Oh WS, Ko KS, Chung DR, Peck KR, et al. Clinical outcomes and risk factors of community-acquired pneumonia caused by gram-negative bacilli. Eur J ClinMicrobiol Infect Dis 2008; 27:657–61.

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