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Research Article

Common Pathogens and Their Resistance to Antimicrobials in Community Acquired Pneumonia (CAP): A Single Center Study in Bangladesh

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Abstract:
Pneumonia is a worldwide, serious threat to health and an enormous socio-economic burden for health care system. Community-acquired pneumonia (CAP) is associated with a significant mortality and morbidity. Knowledge of predominant microbial patterns in CAP constitutes the basis for initial decisions about empirical antimicrobial treatment. The aim of this study was to identify the bacterial etiology of CAP in adult hospitalized patients and to see their antibiotic sensitivity pattern as well as to observe their clinical profile and short term outcome. It was a hospital based prospective observational study. A total of 87 hospitalized patients diagnosed with CAP were enrolled consecutively from the medicine ward of Chittagong Medical College Hospital (CMCH). Sputum for Gram staining, Z N staining, culture sensitivity, blood culture and sensitivity and PCR for *Legionella pneumophila, Mycoplasma pneumoniae, Chlamydophila pneumonia* and *Streptococcus pneumonia* were done. Patients were followed up for in-hospital outcome and 30-day mortality. The mean (±SD) age was 49.59±16.97 years and male female ratio was 1.56:1. Fever, chest pain and cough were the most common clinical features. Sputum culture, blood culture and PCR were positive in 60.9%, 1.1% and 4.6% of the samples respectively. *Klebsiella pneumoniae* was identified in the sputum culture of the majority of the patients (39.1%), followed by *Pseudomonas aeruginosa (10.3%), Staphylococcus aureus and Escherichia coli (5.7%)*. The only one sample which was positive in blood culture and it was *Staphylococcus aureus. Streptococcus pneumoniae* was identified in all the 4 PCR positive cases. The highly sensitive drugs were meropenem, levofloxacin and amikacin. The mean (±SD) duration of hospital stay was 6.34±2.37. In hospital mortality and 30-day mortality was 6.9% and 16.1% respectively. Gram-negative bacteria pre-dominate in the bacteriologic profile of CAP using conventional sputum and blood culture. There is need for further conventional serologic tests for atypical and viral pathogens in all patients admitted with CAP.

Key words: Community Acquired Pneumonia (CAP), Antibiotic resistance, common pathogens

Introduction:

Pneumonia is defined as an acute respiratory illness associated with recently developed radiological pulmonary shadowing which may be segmental, lobar or multi-lobar[1]. Community acquired pneumonia (CAP) is defined as pneumonia acquired outside the hospital by an

immune-competent individual. It is to be distinguished, on the basis of a wider spectrum of pathogens, from nosocomial pneumonia (which arises more than 48 hours after hospital admission or within 3 months of discharge) and from pneumonia in an immune-compromised host (e.g., in the setting of neutropenia, iatrogenic immune-suppression with drugs, status post organ or stem-cell transplantation, HIV infection, or a congenital immune deficiency[2].

Underlying diseases (Chronic obstructive pulmonary disease, compromised immune system, dementia, gastro esophageal reflux disease, etc.) increase susceptibility of the patients for pneumonia[3]. Alternatively, habitual pathogens could show particular patterns of antimicrobial resistance[4]. Undoubtedly the knowledge of these microbiological characteristics is critical and represents the basis for empirical treatments. Serious co-existing illness has been identified as modifying factors of severity of pneumonia[5]. On the basis of these appreciation, published guidelines on pneumonia advocate specific criteria for antibiotic selection and the management of patients in the presence of co-morbid diseases[6].

As the etiology of CAP varies geographically the understanding of local epidemiology may play an important role in making proper empirical treatment choices before laboratory test results are available. This is especially true for Bangladesh and other developing countries where healthcare systems operate on poor hygiene system and lack proper facilities to contain infections. In these countries, early treatment is usually based on the patient's clinical symptoms rather than diagnostic results. Therefore, patient's early prognosis to final outcome might be much improved by available epidemiologic data for the most frequently isolated pathogenic organisms[7]. However, such complete data is scarce in Chittagong, Bangladesh.

There is a need to conduct regular prevalence and antibiogram studies to develop empirical guidelines for treatment of CAP in that particular region. The hospital antibiogram is a periodic summary of antimicrobial susceptibilities of local bacterial isolates submitted to the hospital's clinical microbiology laboratory. Antibiograms are often used by clinicians to assess local susceptibility rates, as an aid in selecting empiric antibiotic therapy, and in monitoring resistance trends over time within an institution. Antibiograms can also used to compare susceptibility rates across institutions and track resistance trends[8].

On the other hand, antimicrobial resistance is a well-known important emerging clinical and public health problem. Controlling antimicrobial resistance is a major issue confronting organized health care today. The irrational use of antibiotics has an influence in the spread of antimicrobial resistance among bacteria[9]. Both under-use and over-use of broad spectrum antibiotics for an infection as common as CAP could be harmful, particularly in Asia where mortality is high, resources scarce and antibiotic resistance an increasing problem[10].

National data on incidence, etiology and mortality of CAP is not available in Bangladesh. Pneumonia's mortality is highly correlated with socio-economic factors and is a far less frequent cause of death in high-income countries. It occurs about five times more frequently in the developing world than the developed world. The incidence of CAP ranged from 4 million to 5 million cases per year, with about 25% requiring hospitalization[11]. Death rates in hospital admitted patients are between 5-10% but may be as high as 50% in severe illness[1]. The annual incidence of CAP in Europe is 1.6-10.6/1,000 adults[12]. In Asia, CAP causes an estimated one million adult deaths per year (160,000 cases per year among those aged 15-59 years)[7].

CAP is a frequent cause of antibiotic prescription, either in hospital or in the ambulatory setting. Antibiotics prescribed for CAP exert a selection pressure favoring the emergence of multidrug resistant bacteria, not only on lung pathogens (like *S. pneumoniae* or *M. pneumoniae*), but also on bacteria colonizing the skin or the gastrointestinal tract, like Staphylococcus aureus or enterobacteriaceae. The emergence of methicillin-resistant *S. aureus*, and lately of extended spectrum betalactamase (ESBL)-producing enterobacteriaceae, are worrying consequences of widespread antibiotic use. A high correlation is present between the prevalence of *S. pneumoniae* resistance towards diverse antibiotic classes and

the density of prescription of the same antibiotic classes. On an individual level, one-time antibiotic administration enhances the prevalence of colonization by resistant bacteria up to one year[13].

In CAP, in approximately 6% of the cases a MDR pathogen is involved, the most frequent described being *S. aureus* and *P. aeruginosa*. A study by Aliberti et al. reported that MDR microorganisms were involved in 3.3% to 7.6% CAP cases in which the most commonly identified MDR pathogen was methicillin-resistant *S. aureus* (MRSA). Community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has become an important CAP pathogen in endemic areas in Europe[14]. Since the recommendation of current international guidelines for severe CAP is empiric therapy with β-lactam with macrolide or fluoroquinolone, which may not provide adequate protection against MRSA, microbiological diagnosis of these cases is very important[11].

To get updated information for proper therapeutic interventions, periodic evaluation and regional surveillance programs is necessary for the development local data about the antimicrobial sensitivity to respiratory pathogens for CAP. In this study, we aimed to identify the common bacterial pathogens in hospitalized patients with CAP from Chittagong, Bangladesh. We also determined pathogens' antibiotic susceptibility patterns to evaluate the changing trend of antimicrobial susceptibility in this region. Meanwhile the short term clinical outcomeof antibiotic therapy for CAP during hospital stay was also observed.

Though hospitalization of adult patients with CAP are increasing in Bangladesh, information regarding their clinical presentation, microbiological characteristics, antimicrobial susceptibility pattern that is required for choosing empiric antibiotic treatment and outcome of patients are lacking. Microorganisms causing CAP vary in their susceptibility to antimicrobials from place to place and time to time. CMCH is the 2nd largest tertiary care hospital in Bangladesh and a referral centre to the hospitals in the surrounding districts. Up to date knowledge of the microbial organisms and antibiotic susceptibility pattern of patients with CAP is essential for defining empirical treatment. There is a paucity of recent study regarding these issues in Bangladesh especially in peripheral hospital settings, where a large number of CAP patients with different comorbidities are managed routinely.

Methodology:

The hospital based prospective observational study was conducted from August 2018 to July 2019 in the Department of Medicine at Chittagong Medical College Hospital. Patients of both sexes age above 18 years who were diagnosed as CAP admitted in the Department of Medicine was included in the study as consecutive sampling method. Total 85 admitted patients were included after screening of exclusion criteria like chemical pneumonitis, malignancy, radiological evidence of fibrosis, collapse, bronchiectasis, lung abscess and tuberculosis, suspicion of immunosuppression or known immunosuppressive status like HIV, Hematological or lymphoid malignancy. Patient on immunosuppressive drugs- steroids and chemotherapy and getting antibiotic for more than 48 hours were also excluded.

Prior approval for the study was taken from the institutional ethical review committee of Chittagong Medical College. Patients diagnosed clinically as CAP were enrolled in the study. Investigations like hematologic measurements (TC of WBC, Hb%, ESR), blood culture, chest X-ray P/A view, sputum for Gram staining and culture sensitivity, sputum for AFB for 3 consecutive samples, blood urea and random blood sugar were done. For scanty production of cough, patient's sputum was collected after nebulization by hypertonic normal saline. Patient with positive radiological findings of consolidation was enrolled in the study. During treatment, oral temperature was recorded and frequently physical examinations were performed up to discharge. Patients were asked to report 30 days after discharge for follow up.

Microbiological laboratory tests: Blood and Sputum samples were collected from all patients enrolled in the study. Sputum originated from the lower respiratory tract were defined as that containing >25 granulocytes and <10 epithelial cells per low power field microscopic view. Validated sputum was cultured in blood agar, chocolate agar and McConkey's agar media. Primary blood cultures were done in Trypticase

soya broth and secondary blood cultures were done on blood agar, Chocholate agar and McConkey's agar media.

Sputum microscopy and culture: Purulent part of sputum were collected and a thin smear were made then Gram staining and microscopy were done. Specimens were classified by Bartlett's Criteria; Bacterial morphological types were screened at oil immersion field.

Blood agar media was used for primary isolation and study of haemolytic property of the organism, Chocolate agar media for isolation of fastidious organisms and MacConkey agar media for isolation of Gram negative organisms.

Antimicrobial agents used (CLSI 2017): Most of the commonly used antimicrobials were tested for susceptibility for Gram positive and Gram negative cocci and diplococci with Meropencm (10 microgram), Ceftriaxone (30 microgram), Amoxyclav (30 microgram), Levofloxacin (5 microgram), Azithromycin (1 microgram), Cefixime (30 microgram), Vancomycin (30 microgram) and Amikacin (10 microgram).

PCR was done for S*treptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila.*

Statistical analysis was done using SPSS- version 23. Continuous variables were reported as means and standard deviation and categorical variables were reported as count and percentages. Between groups comparisons were done either by Chi square test or Fisher exact test for categorical data. Statistical significance was defined as $P < 0.05$ and confidence interval set at 95% level.

Results:

Table I: Age and sex distribution of the 87 admitted patients with CAP

Age (Years)	Total	Male	Female
<40 years	26 (26.9%)	14 (53.8%)	12 (46.2%)
40-59 years	31 (35.6%)	21 (67.7%)	10 (32.3%)
≥60 years	30 (34.5%)	18 (60.0%)	12 (40.0%)
Total	**87 (100%)**	**53 (60.9%)**	**34 (39.1%)**
Mean ± SD	49.59±16.97	50.70±16.32	47.85±18.05
Range	18-76	18-75	19-76

The mean (±SD) age was 49.59±16.97years with ranged from 18 to 76 years and maximum number (35.6%) of patients was found in the age group of 40-59 years. There was male predominance with a male to female ration of 1.56:1 (Table I). Different laboratory findings of the enrolled CAP patients show that, sputum gram stain was positive in 55 (63.2%) patients while Z-N stain was negative in entire sample. Sputum culture yield growth in 53 (60.9%) sample while blood culture only in 1 (1.1%) sample. PCR was positive in 4 (4.6%) sample.

Table II: Bacteriological profile of the 87 CAP patients by different tests

Test and type of organisms		Frequency (%)
Sputum culture		
	Klebsiella pneumonia	34 (39.1%)
	Pseudomonas aeruginosa	9 (10.3%)
	Staphylococcus aureus	5 (5.7%)
	Escherichia coli	5 (5.7%)
Blood culture		
	Staphylococcus aureus	1 (1.1%)
PCR		
	Streptococcus pneumonia	4 (4.6%)

Klebsiella pneumoniae was identified in the sputum culture of the majority of the patients (39.1%), followed by *Pseudomonas aeruginosa (10.3%), Staphylococcus aureus and Escherichia coli (5.7%)*. The only one sample which was positive in blood culture and it was *Staphylococcus aureus. Streptococcus pneumoniae* was identified in all the 4 PCR positive cases.

In hospital mortality rate of the CAP patients in this study was 6.9% and 30-day mortality rate was 14.1%. Average length of hospital stay was 6 days. About one tenth of the total patients develop sepsis and need ICU support. Patients who died within 30 days, majority had either

Klebsiella pneumonia or Pseudomonas aeruginosa. Among survivors in addition of these two organisms *Staphylococcus aureus, Escherichia coli* and *Streptococcus pneumonia* were identified. There was some variation in the distributions of the isolated organisms in different age groups. *Pseudomonas aeruginosa* were more prevalent in CAP patient's age ≥60 years. In contrast no *Escherichia coli* was identified among this age group.

Proportion of the patients having no organisms detected in the testes were almost equal in male and female patients (35.8% versus 35.3%). The distributions of the isolated organisms in male and female were also more or less similar.

During admission severity of pneumonia was assessed by CURB-65 score. Patients with severe disease (CURB-65 >2) and with less severe disease (CURB-65 ≤2) have almost similar bacteriological pattern.

Table III: Resistant pattern of the isolated organisms in 87 patients of CAP

Name of antibiotic	Name of organisms isolated from sputum culture			
	K. pneumonia (n=33)	*P.aeruginosa* (n=9)	*S. aureus* (n=5)	*E. coli* (n=5)
Amoxicillin-Clavulanate	27 (81.8%)	9 (100%)	4 (80.0%)	3 (60.0%)
Clarithromycin	21 (63.6%)	6 (66.7%)	5 (100%)	2 (40.0%)
Azythromycin	10 (30.3%)	0 (0%)	5 (100%)	0 (0%)
Vancomycin	NP	NP	1 (20.0%)	NP
Meropenem	1 (3.0%)	0 (0%)	1 (20.0%)	0 (0%)
Trimethoprim-sulphamethoxazole	15 (45.5%)	3 (33.3%)	3 (60.0%)	2 (40.0%)
Ceftazidime	20 (62.5%)	0 (0%)	4 (80.0%)	2 (40.0%)
Ceftriaxone	12 (36.4%)	5 (55.6%)	2 (40.0%)	1 (20.0%)
Cefuroxime	22 (66.7%)	9 (100%)	4 (80.0%)	2 (40.0%)
Cefixime	23 (69.7%)	9 (100%)	4 (80.0%)	2 (40.0%)
Levofloxacin	2 (6.1%)	0 (0%)	1 (20.0%)	2 (40.0%)
Amikacin	0 (0%)	1 (11.1%)	1 (20.0%)	0 (0%)

NP: Drug not in panel.

K. pneumoniae were highly resistant to Amoxicillin-Clavulanate, Cefixime, Cefuroxime, clarithromycin and Ceftazidime (81.8%, 69.7%, 66.7%, 62.5% and 63.6% respectively). *P. aeruginosa* were highly resistant to Amoxicillin-Clavulanate, Cefixime, Cefuroxime and clarithromycin (100%, 100%, 100% and 66.7% respectively). *S. aureus* were highly resistant to clarithromycin, azytyhromycin, Amoxicillin-Clavulanate, Ceftazidime and Cefixime (100%, 100%, 80% and 80% respectively).

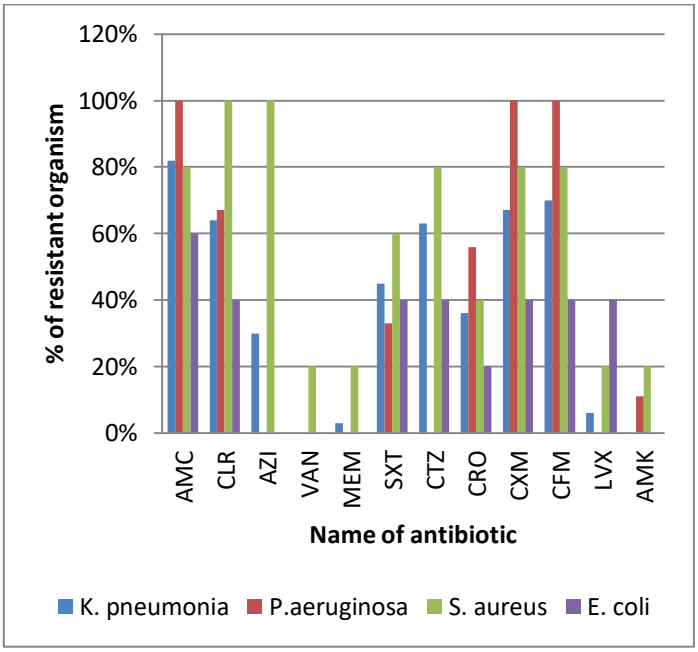

Figure 1: Resistant pattern of the isolated organisms in 87 patients of CAP

AMC= Amoxicillin-Clavulanate, CLR= Clarithromycin, AZI= Azithromycin, VAN=

Vancomycin, MEM= Meropenem, SXT= Trimethoprim-sulphamethoxazole, CTZ= Ceftazidime, CRO= Ceftriaxone, CXM= Cefuroxime, CFM= Cefixime, LVX= Levofloxacin, AMK= Amikacin

Table IV: Overall sensitivity pattern of the tested organisms

Name of antibiotic	N	Sensitivity pattern		
		Resistance	Intermediate sensitive	Sensitive
Amoxicillin-Clavulanate	52	43 (82.7%)	1 (1.9%)	8 (15.4%)
Clarithromycin	52	34 (65.4%)	13 (25.0%)	5 (9.6%)
Azythromycin	52	15 (28.8%)	7 (13.5%)	30 (57.7%)
Vancomycin	5	0 (0%)	1 (20.0%)	4 (80.0%)
Meropenem	52	2 (3.8%)	0 (0%)	50 (96.2%)
Trimethoprim-sulphamethoxazole	52	23 (44.4%)	4 (7.7%)	25 (48.1%)
Ceftazidime	51	26 (51.0%)	6 (11.8%)	19 (37.2%)
Ceftriaxone	52	20 (38.5%)	2 (3.8%)	30 (57.7%)
Cefuroxime	52	36 (69.2%)	7 (13.5%)	9 (17.3%)
Cefixime	52	38 (73.1%)	3 (5.8%)	11 (21.2%)
Levofloxacin	52	5 (9.6%)	1 (1.9%)	46 (88.5%)
Amikacin	52	0 (0%)	2 (3.8%)	50 (96.2%)

Overall the isolated organisms in the study were found to be highly sensitive for Meropenem (96.2%), Amikacin (96.2%), Levofloxacin (88.5%) and Vancomycin (80.0%).

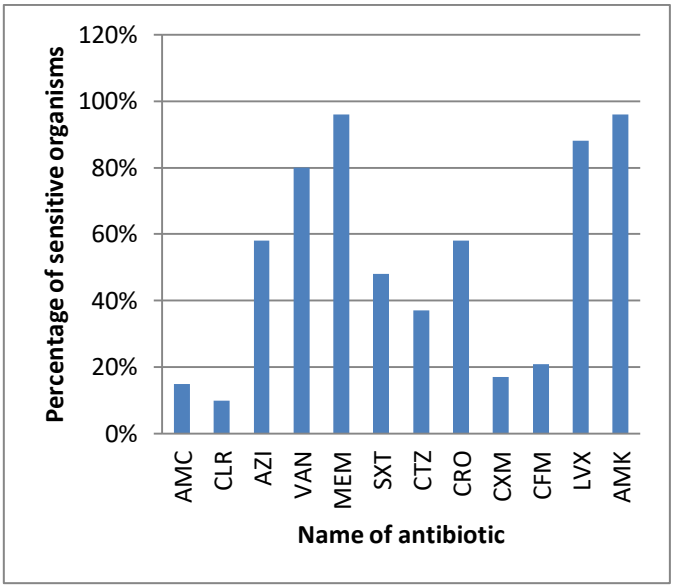

Figure 2: Overall sensitivity pattern of the tested organisms.

AMC= Amoxicillin-Clavulanate, CLR= Clarithromycin, AZI= Azithromycin, VAN= Vancomycin, MEM= Meropenem, SXT= Trimethoprim-sulphamethoxazole, CTZ= Ceftazidime, CRO= Ceftriaxone, CXM= Cefuroxime, CFM= Cefixime, LVX= Levofloxacin, AMK= Amikacin

Discussion:

This hospital based prospective observational study was conducted to determine the clinico-bacteriological profile and in-hospital outcome of hospitalized adult patients with CAP. For this purpose, 87 patients of CAP who had clinical and radiological features of pneumonia and were admitted in different medicine wards of CMCH were enrolled in the study.

The microbial diagnosis of CAP was confirmed in 65.5% of patients with standard sputum culture, blood culture and PCR test (53/87 were sputum culture positive and 4/87 were PCR positive). However, this rate varies in different studies. With different laboratory testing the etiological diagnosis could be confirmed in 29%, 49% and 75.6% in the studies of Naik et al, Salam et al, and Bansal et al, respectively[15,16,17]. Comparatively

high incidence of the etiological diagnosis in the present study is probably explained by the strict inclusion criteria. Patients with a history of getting antibiotic for more than 48 hours were excluded from the present study. Studies like Naik et al have comparatively relaxed inclusion criteria and included patients irrespective of their duration of antibiotic history and consequently a lower yield of organisms was found. However, no causative organisms were identified in a significant proportion of patients (34.5%) in the present study. The possible causes for the inability to determine etiology in these patients were lack of sensitivity of laboratory investigations, prior antibiotic treatment and lack of more sophisticated investigations which are expensive and require highly trained personnel. Other prospective studies for evaluating the causes of CAP in adults have failed to establish an etiologic diagnosis in 40 - 60% of cases even with extensive diagnostic testing[18,19].

The most common organism isolated from sputum culture was *Klebsiella pneumoniae* followed by *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* However, globally, *Streptococcus pneumoniae* (pneumococcus) is widely accepted as being the most common pathogen in CAP, usually presented with acute symptoms of lower respiratory tract infection. An estimated prevalence of 19.3% to 34% was reported for *S. pneumoniae* in Europe[20,21]. A previous study conducted in another tertiary care hospital of Bangladesh (Salam et al., 2016) *Streptococcus pneumoniae* was the most frequent organism isolated from the sputum culture from the hospitalized patients with CAP. Though *S. pneumonia* is commonest organism reported globally, but some studies, conducted in last two decades from neighboring countries in South Asia have reported higher incidence of gram-negative organisms among culture-positive pneumonias. Studies from Malaysia reported that, gram-negative bacteria, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, were the most common causes of CAP in their series[18]. Another study from India reported that, *Klebsiella pneumoniae* (42.85%) was the most common followed by *Pseudomonas aeruginosa* (28.57%), *Staphylococcus aureus* (21.43%) and *Streptococcus pneumoniae* (7.14%)[22]. Mythri et al. reported that the most common pathogen was *Klebsiella spp* followed by *S. pneumoniae* and *P. aeruginosa*. These findings indicate that, CAP by Gram-negative organisms constitute a significant burden in our locale and the spectrum of organisms is subject to geographical alternations[23]. However, compared with western studies, *S. pneumonia* was of less relative importance in Asian countries. Gram-negative bacilli and Mycobacterium *tuberculosis* were more important in this continent.

To increase the microbiological diagnosis in the present series PCR was done to detect *Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila* and *Streptococcus pneumoniae.* However, only 4 samples yield positive result and all of them were *Streptococcus pneumoniae.* Intracellular pathogens are frequent causes of pneumonia, in these cases the clinical presentation is 'atypical', characterized by sub–acute symptoms, non-productive cough, low fever, normal white blood cells count and with frequency associated extrapulmonary manifestations. However, the incidence is variable; depending in part on the difficulties with microbiological cultures they grow poorly in standard culture media and culture requires expertise[24].

It was observed from this study that isolated *Klebsiella* strain was mostly resistant to commonly used antibiotics for CAP like Amoxicillin-Clavulanate, Cefixime, Cefuroxime, clarithromycin and Ceftazidime. *P. aeruginosa* were highly resistant to Amoxicillin-Clavulanate, Cefixime, Cefuroxime and clarithromycin. *S. aureus* were highly resistant to clarithromycin, azytyhromycin, Amoxicillin-Clavulanate, Ceftazidime and Cefixime. Other isolated organisms like Pseudomonas, *Escherichia coli*, were also resistant to B-lactamase inhibitor, Macrolides and third generation cephalosporin. This study also revealed Meropenem, Amikacin and levofloxacin were the most sensitive antibiotics for the organisms identified form the CAP patients. However, Meropenem is costly and not recommended by the guideline published by American thoracic society (2004) and Infectious disease society of America (IDSA 2004)[25].

Frequent use beta-lactam antiobiotic and Macrolides for the treatment CAP are first line

regimens but emerging strain are more resistant to these conventional antibiotics. Multi drug resistant to beta-lactamase, Macrolides and Fluroquinolone is an emerging problem and complicating the management of CAP[26]. Alarming outcome of resistant bacteria was also observed in a study conducted by Salaam et al in Dhaka Medical College Hospital.

The present study was conducted over a short period of just over nine months and it is possible that less common pathogens were not detected during the study. A larger multi-center study is needed to obtain accurate information on the epidemiology of CAP in the country. Moreover, the etiology remained undetermined in 21.4% of patients who died during hospitalization. This emphasizes the need of further investigations in patients in whom the bad prognostic factors are present at the time of admission so as to establish the etiology, start early treatment and thereby reducing mortality.

Conclusion:

In conclusion, we found that the Gram-negative bacilli like *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* were common organism for CAP identified by sputum culture. *Staphylococcus aureus* was found by PCR test. For CAP that required hospitalization sensitivity results were in favor of Meropenem, Amikacin and Levofloxacin. Overall mortality in hospital and 30 day were high.

Regional differences in bacteriological profile as well as their sensitivity pattern should be considered during selecting the best and sensitive drugs for treating CAP. To determine the full etiological spectrum of CAP future studies incorporating large sample with serologic tests for atypical and viral pathogens from different center is essential.

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