Research Article

Clinical Outcome and *In-vitro* Microbiological Response of Bacterial Isolates to Commonly Prescribed Antibiotics among Hospitalized Patients with Community Acquired Pneumonia in Jimma University Specialized Hospital, Ethiopia

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ABSTRACT

Background: Initial antibiotic treatment for community acquired pneumonia (CAP) in Ethiopian settings is invariably empirical and further clinical decision making upon inadequate initial response is not evidence-based. A detailed knowledge of the local susceptibility pattern of the pathogens would ensure a more appropriate and evidence-based selection of the initial antibiotic(s). This study was conducted to assess the clinical outcome and *in-vitro* response of bacterial isolates to locally available and commonly prescribed antibiotics among hospitalized adults with community acquired pneumonia at JUSH. **Materials and Methods:** A prospective observational cohort study was conducted on sixty hospitalized patients. Clinical outcome including responding pneumonia, non-responding pneumonia, death, or progressive pneumonia was assessed using clinical parameters. *In-vitro* microbiological response of the bacterial isolates was determined to prescribed antibiotics (doxycycline, 30 μg and ceftriaxone, 30μg) using disk diffusion and Stock methods. **Results:** Of all patients with CAP (n = 60) two species of potential bacterial causes of pneumonia were isolated; *S. pneumoniae* accounted for 19(57.6%) while *S. aureus* accounted 14 (41.7%). *S. pneumoniae*, 6 (31.6%) were resistant to doxycycline and 4 (21.1%) of the isolates were resistant to ceftriaxone. Half of the *S. aureus* isolates were susceptible to doxycycline while 3 (21.4%) were resistant to this antibiotic. Half, 50%, of *S. aureus* isolates were resistant to ceftriaxone. Clinically 25% of the participants had non-responding pneumonia, however, 45 (75%) had responding pneumonia to the combined therapy of doxycycline and ceftriaxone. The presence of co-morbid illness was associated with inadequate initial clinical outcome ($p = 0.03$). **Conclusion:** *S. pneumoniae* was the common isolate and showed high resistance rate to both ceftriaxone and doxycycline. One fourth of the patients experienced non-responding pneumonia. The presence of co-morbid illnesses was significantly associated with inadequate initial clinical response.

Key words: CAP, Microbiological response, Clinical outcome, Non-Responding pneumonia, Responding pneumonia.

INTRODUCTION

The involvement of multiple drug resistant (MDR) pathogens has led to revised classification system of pneumonia in which infection is categorized as either community-acquired pneumonia (CAP) or health care–associated pneumonia (HCAP).[1,2] *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus*, and atypical organisms are among the common cause of CAP. *S. pneumoniae* accounts for about 50% of all cases of CAP requiring hospital admission;[3-6] and up to 76% cases of CAP bacterial pneumonia.[7] The spectrum

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of etiologic agent and the initial approach to therapy is influenced majorly by the severity of initial presentation and the presence of the co-morbid illness or advanced age.[8]

CAP continues to be an important public health problem worldwide with a mortality rate of 8%-15%;[9] and complications in 15% to 50% of hospitalized patients.[10] The incidence of treatment failure (TF) in CAP is 10 to 15%, and the mortality is increased nearly fivefold. The three causes of treatment failure are resistance, unusual microorganisms and noninfectious. Risk factors for treatment failure are related to the initial severity of the disease, the presence of comorbidity, the microorganism involved, and the antimicrobial treatment implemented.[11]

Resistance in CAP is being identified with increasing frequency among *S. pneumoniae, Hemophilus influenzae,* and a number of enteric gram-negative bacteria.[12] Worldwide all surveillance studies are continually report increasing *in-vitro* resistance to antimicrobial agents including penicillin, second generation cephalosporins, macrolides, tetracyclines, and trimethoprim-sulfamethoxazole.[13] Initial antimicrobial therapy is normally given empirically and in many cases treatment is empirical throughout due to the lack of reliable microbiological data. An understanding of the possible pathogens and resistance patterns is helpful in guiding antibiotic choice, and a detailed knowledge of the local susceptibility of the potential pathogens would ensure a more appropriate selection of the antimicrobial agent to be used.[14]

Ethiopia is one of the top 15 countries with the highest estimated number of deaths due to clinical pneumonia in children under 5 years.[15] Initial antibiotic treatment for community acquired pneumonia in Ethiopian settings is invariably empirical and further clinical decision making upon inadequate initial response is not evidence-based. In setups where the diagnosis isnot supported by confirmation of the specific etiologic agent(s), information on local susceptibility to antibiotics is of paramount importance for the optimal empirical treatment of patients. Therefore this study tries to bridge this gap by providing information that can be used locally for evidence based patient care. Therefore the findings of this study can be used for development of locally useful protocols and guidelines for the management of CAP.

MATERIAL AND METHODS

Prospective observational cohort study was conducted on sixty hospitalized adults hospitalized with community acquired pneumonia at Jimma University Specialized Hospital medical wards, South-West Ethiopia (Ethiopia) from February 1 to April 30, 2013. Patients hospitalized with the diagnosis of community acquired pneumonia, hospital stay of less than 48 hours, and with age of ≥15 years were included. Patients on antibiotic therapy before the sputum specimen collection, recently transferred from other health care facility, those who could not expectorate the sputum specimen for culture and patients died before treatment were exclude from the study. Socio-demographics characteristics of patients, clinical characteristics, the clinical outcome and the *in-vitro* microbiological response of the bacterial species to the prescribed antibiotics were collected. Clinical data was collected from the patient medical record, reviewing the chart and through patient interview. The patients were followed prospectively throughout the hospital stay.

Assessment of Clinical Outcome

The clinical outcome including responding pneumonia, non-responding pneumonia, death and progressive pneumonia was assessed using clinical parameters. The patients were followed two medical doctors prospectively throughout the hospital stay and the clinical outcome was assessed for the commonly prescribed antibiotics in the setting (Ceftriaxone 1 gram twice daily administered intravenously and Doxycycline 100mg adminstered orally twice daily). Responding pneumonia was defined as clinical stability during the first 72 hours (Heart rate <100 beats/min, systolic blood pressure ≥90 mmHg, respiratory rate <24 breaths/min and Temperature <37.2c^0).Progressive pneumonia refers to clinical deterioration atleast after 72 hrs of treatment; and non-responding pneumoniarefers to absence of clinical response upto day 5 day of treatment.

In-Vitro antimicrobial Susceptibility Testing

The sputum was collected in a sterile wide-mouthed screw caped container after instructing the patient to rinse his/her mouth thoroughly with water and cough deeply to produce a sputum specimen. Gram staining was done from purulent or mucopurulent part of sputum. The sputum was inoculated on blood agar and chocolate agar and incubated at 35–37^{o}C over 24 hours in atmosphere containing extra carbon dioxide (in a candle jar). Mannitol salt agar (MSA) plate was used to selectively support the grow of *S. aureus*. Colony characteristics, zones of haemolysis, catalase test, optochin sensitivity (5µg) and bile solubility test were used to identify S. *pneumoniae*. Chocolate agar, enriched with factor V (NAD) and

factor X (hemi) was used to enhance the growth of *H. influenza*. *S. aureus* species were confirmed by catalase, coagulase and the mannitol fermentation tests.[16-17] pure bacterial suspension of turbidity comparable to McFarland 0.5 standard was inoculated on Muller Hinton agar and the susceptibility was done by disk diffusion and Stock method. The susceptibility testing was done against the following antimicrobial drug with stated concentration: ceftriaxone (30 μg) and doxycycline(30 μg). The result was interpreted based on the zone of inhibition as susceptible, intermediate or resistant as per specified in the guide line for the individual antibiotics.[18-19] All microbiological procedure starting from specimen collection to sensitivity test were conducted by two medical microbiologists.

Ethics

The current study was conducted after approval was obtained from the ethical review board of Jimma University. Written consent was secured from study participants before recruitment.

Statistical methods

The data was cleaned, entered into and analyzed using SPSS for windows version 20 of the computer software. Descriptive statistics; chi-square or Fischer's exact tests was used for data analysis and interpretation. All reported p-values were two-tailed.A $P<0.05$ was considered statistically significant.

RESULTS

A total of60 patients hospitalized with community acquired pneumonia were included in the study. The median age of patients was 45 years with range of 15-73 years as shown in Table 1 below.

The main clinical characteristics of the patients are summarized in Table 2. Nearly, two third, 41(68.3%) of study patients had co-morbid illnesses. Half of study participants had British thoracic society score (BTS) pneumonia severity score class III; while 29(48.3%) had BTS pneumonia severity score class II. All of the patients were treated as inpatients with combination of doxycycline (100mg twice daily taken orally) and ceftriaxone (1 gm twice daily administered intravenously). There were 2(3.3%) participants with previous history of hospitalization within three months. Previous history of antibiotics medication was reported among 7(11.7%) of study participants.

Table 1: Frequency distribution of the Socio-demographic characteristics hospitalized patients with community acquired pneumonia in JUSH medical wards, February 1 to April 30, 2013

Variables	N (%)
Sex	
Male	34(56.7)
Female	26(43.3)
Age(in year),median & range	45 ,[15-73]
Age category (in year)	
<40	22(36.7)
40-49	11(18.3)
≥50	27(45)
Marital status	
Married	51(85)
Single	8(13.3)
Divorced	1(1.7)
Widowed	0(0)
Education level	
Illiterate	48(80)
Elementary	10(16.7)
High school	1(1.7)
University or college	1(1.7)
Occupation	
Farmer	53(88.3)
Merchant	4(6.7)
Civil Servant	2(3.3)
Any other	1(1.7)
Smoking status	
Smoker	4(6.7)
Non-smoker	56(53.3)
Alcoholism use	
Yes	4(6.7)
No	56(53.3)

In-Vitro Microbiological Responses

The rate of isolation of bacterial species from the sputum culture was 50%. The frequency of distribution of organisms isolated from the sputum culture was depicted in Table 3.

Only two potential bacterial causes of pneumonia were isolated; *Streptococcus pneumoniae* accounted for 19(57.6%) while *Staphylococcus aureus* accounted for 14 (41.7%) of the two pathogens. Both *S. pneumoniae* and *S. aureus* isolates were isolated from three patients.

Nearly, two third, 13 (68.4%) of *S. pneumoniae* were susceptible to both drugs while 6 (31.6%) were resistant to doxycycline (30 μg). about 4 (21.1%) *S. pneumoniae* isolates were resistant to Ceftriaxone while 15(78.9%) were susceptible. Half of the *S. aureus* isolates were susceptible to doxycycline but 4 (28.6%) had intermediate resistant while 3(21.4%) were resistant. Half of *S. aureus* isolates were resistant to Ceftriaxone as it was summarized in Table 4.

Table 2: Frequency distribution of the clinical characteristics of hospitalized patients with community acquired pneumonia in JUSH medical wards February 1 to April 30, 2013

Variable	N (%)
Hospital admission within three months	
Yes	2(3.3)
No	58(96.7)
Antibiotic use within three months	
Yes	7(11.7)
No	53(88.3)
Comorbid disease	
Present	41(68.3)
Absent	19(31.7)
Pneumonia severity class (BTS/score)	
II	29(48.3)
III	30(50)
IV	1(1.7)
WBC count(/mm^3)	
$4*10^3$	9(28.1)
$4*10^3$-$10*10^3$	8(25)
$>10*10^3$	15(46.9)

WBC= white blood cell count; N=frequency; %=percentage.

Table 3: Frequency distribution of the bacterial species isolated from sputum culture of hospitalized patients with community acquired pneumonia in JUSH medical wards February 1 to April 30, 2013

Variable	N (%)
Bacterial identification	
Yes	30(50)
No	30(50)
Identified bacterial species	
Streptococcus pneumoniae	19(58.3)
Staphylococcus aureus	14(41.7)
S.pneumoniae and S.aureus	3(6%)

N= Frequency, %=percentage.

Table 4: The Frequency distribution of susceptibility pattern of the bacterial species isolated from sputum culture of hospitalized patients with community acquired pneumonia in JUSH medical wards February 1 to April 30, 2013

Antibiotics	Susceptibility Pattern Number (n) & Percent (%)		
	Susceptible(s)	Intermediate(I)	Resistant(R)
Doxycycline(DO), 30 µg			
Streptococcus pneumoniae	13 (68.4)	0(0.0)	6(31.6)
Staphylococcus aureus	7(50.0)	4(28.6)	3(21.4)
Ceftriaxone(CRO), 30 µg			
Streptococcus pneumoniae	15(78.9)	0(0.0)	4(21.1)
Staphylococcus aureus	7(50.0)	7(50)	0(0.0)

Clinical Outcome and Associated Factors

On clinical evaluation, 45 (75%) of participants had responding pneumonia, however, 15 (25%) of them had initially non-responding pneumonia to the combination of doxycycline and ceftriaxone therapy. The majority of non-responders were male, (73.3%).One third, 5(33.3%) of non-responder were aged less than 40 years; 4 (26.6%) were 40-49 years age range while 6(40%) of them were ≥50 years of age. Of the responders, 17(37.7%) were < 40 years; 7(15.5%), 40-49 range of years; and 21(46.7%) accounts for age ≥ 50 years (Table 5).

Co-morbid illness was present in 13(86.7%) of the non-responders and in 28(62.8%) of responders to treatment. One third of non-responders had the BTS pneumonia

Table 5: The association of clinical outcome and related variable among hospitalized patients with community acquired pneumonias in JUSH medical wards February 1 to April 30, 2013

Variable	Clinical outcome (n (%))		P-value	95%CI
	RP	NRP		
Sex			0.23	[0.11,1.34]
Male	23(67.6)	11(32.4)		
Female	22(84.6)	4(15.4)		
Age			0.65	[0.53 , 0.77]
<40	17(77.3)	5(22.7)		
40-49	7	4		
≥50	21 (77.8)	6 (22.2)		
Co-morbid illness				[0.01, 0.98]
Present	28(69.3)	13(31.7)	0.03	
Absent	17(89.5)	2(10.5)		
BTS score(CRB-65)			0.133	[0.00, 0.08]
II	24(82.6)	5(17.4)		
III	21(70.0)	9(30)		
IV	0	1		
Smoking			0.26	[0.04, 2.36]
Smoker	2	2		
Non-smoker	43(76.8)	13(23.2)		
Alcoholism			0.26	[0.04, 2.36]
Yes	2	2		
No	43(76.8)	13(23.2)		
Antibiotic use within 3 months			0.82	[0.14, 4.70]
Yes	5	2		
No	40(75.5)	13(24.5)		
Hospitalization within 3 months			0.44	[0.02, 5.43]
Yes	1	1		
No	44(75.9)	14(24.1)		
Identified bacterial isolate			0.27	[0.95, 1.00]
S. *pneumoniae*	15(78.9)	4(21.1)		
S. *aureus*	12(85.7)	2(14.3)		
***S. pneumoniae* susceptibility to doxycycline(DO, 30 µg)**			0.58	[0.28, 26.61]
Susceptible	11(84.6)	2(15.4)		
Resistant	4	2		
***S. pneumoniae* susceptibility to ceftriaxone(CRO, 30 µg)**			0.18	[0.56, 76.18]
Susceptible	13(68.7)	2(31.3)		
Resistant	2	2		
***S. aureus* susceptibility to doxycycline((DO, 30 µg))**			0.25	[0.14, 0.36]
Susceptible	7	0		
Intermediate	3	1		
Resistant	2	1		
***S. aureus* susceptibility to ceftriaxone(CRO, 30 µg)**			0.47	[0.878 , 2.27]
Susceptible	7	0		
Intermediate	5	2		

Note! RP= responding pneumonia, NRP= Non-responding pneumonia, n=number, %=percent

severity score class II, while 9(60%) had class II, and one was class with IV. The BTS pneumonia severity score of responders to treatment was: 24(53.3%) class II and class III accounted 21(46.7%) (Table 5).

A total of 6 of bacterial isolates were isolated from those with non-responding pneumonia: *S. pneumoniae* (n=4) and S. *aureus* (n=2). The proportion of bacterial isolates among the responders was: *S. pneumoniae* (n=15*)* and *S. aureus* (n=12). Of the six drug resistant *S. pneumoniae* isolates to doxycycline; two were from non-responders while four of them were isolated from those with responding pneumonia (Table 5).

The presence of co-morbid illness were statistically associated with clinical outcome (p value=0.03). Although resistant potential bacterial isolates causes for CAP were identified from non- responders the association of *in-vitro* microbiological response with clinical outcome was not statistically significant with (p>0.05).

DISCUSSION

Potential pathogenic bacteria isolates were found among half of patients hospitalized with community acquired pneumonia from the sputum culture. The rate of isolation was similar to other studies from India with isolation rate of 47.7% but lower than that the finding of Shilma *et.al*, in the same country 75.6%.[3,8,20-21] The source of difference is explained that in this study atypical microorganism, gram negative bacteria other than *Haemophilus influenzae* and viral etiologic agents were not identified.

This study showed that *S. pneumoniae* was the commonest potential organism causing CAP similar to the report from other countries.[22-24] *S. aureu*s was isolated as second potential cause of CAP. The incidence of *S. aureus* in CAP can be explained by spread of staphylococcus from hospital setting to community and *staphylococcus* complicating virus illnesses.[8] In some studies, participants with tuberculosis, HIV/AIDS and on immuno suppressive therapy were excluded but not in the current study.

The prevalence of *S. pneumoniae* resistance rate to both todoxycycline and ceftriaxone in the current study was higher than finding of other studies. Similar study done in Kenya reported resistance rate of lower resistance rate (24.2%) from outpatients.[25] Study done in Japan showed the following pattern: susceptible, (96.5%); intermediate, (2.8%) and resistant, (0.7%) to ceftriaxone.[22] The difference may be due to the fact that half of the patients were from outpatient department. Randomized study done in US on 41 adults hospitalized with CAP showed of 27 *S. pneumoniae* isolates 24 (88.9%) were susceptible to ceftriaxone while 3 (11.1%) of them were resistant to this antibiotic.[26] Study done in Hawassa Referral Hospital (Ethiopia) found of 31 *S. pneumoniae* isolates among clinically diagnosed 152 cases: pneumonia, meningitis and otitis media from sputum, cerebrospinal-fluid, and ear discharge samples culture isolates 22 (71.0%) were susceptible and 9 (29.0%) were resistant to ceftriaxone (30µg).[27] There is difference between the two studies in type of cases, age group of study participants; and specimen source being from participants treated as inpatients in the current study.

The prevalence of *S. aureus* resistance to both doxycycline and to ceftriaxone was lower than previous studies. According to study from Nigeria, among one hundred *S. aureus* ß-lactamase producing strains obtained from different clinical specimen's 30% wereresistant to ceftriaxone; and 70% these isolates were resistance to tetracyclines. Only 5% of non ß-lactamase producing strains were resistant to ceftriaxone while 65% were resistant to tetracycline.[28] The source of difference can be the source of the specimen; it was taken from different clinical specimens other than sputum but in the current study it from only sputum of participants hospitalized with CAP. The difference may be also due to *in-vitro* activity difference between tetracycline and doxycycline.

A study with similar design to current study done in Brazil onadults hospitalizedwith CAP showed that out of8 *S. aureus* isolates, 6 (75.0%) were susceptible, while 2 (25.0%) of them were resistant to tetracycline.[29] In this study participants with tuberculosis and Human Immunodeficiency Virus (HIV) infection were excluded;and there may be *in-vitro* activity difference between tetracycline and doxycycline.

In this study significant proportion participants had initial clinically non-responding pneumonia. Prospective cohort study done in Spain on 1424 hospitalized adults with CAP found that treatment failure was observed in 215 (15.1%) patients.[30] This study differs from the current study by exclusion of immunosuppressed patients; and use of other empirical antibiotic therapy for some patients. Another study randomized study done in US of 573 of hospitalized adult patients with CAP treated with ceftriaxone plus 2 doses of oral clarithromycin 77.7% of achieved clinical cure.[31] This study differs from the current study by use of clarithromycin instead of doxycycline.

In this study there was statistically significant association

between clinical outcome co-morbid conditions. This findings is Similar, to study done in Israel among hospitalized adults with CAP.[32] Other study done in Spain showed com-morbid illnesses were among the factors associated with treatment failure.[10]

In conclusion this study showed significant proportion of initial clinically non-responding pneumonia. Significant proportion of *S. pneumonia* isolates had high *in-vitro* resistance rate to both ceftriaxone and doxycycline. *S. pneumoniae* was the most common bacterial isolate identified as the potential causes of community acquired pneumonia among hospitalized patients with community acquired pneumonia. The presence of co-morbid illnesses was significantly associated with inadequate clinical response. Although the *in-vitro* microbiological resistance was high, it was not statistically significantly associated with the clinical response.

To the best of our knowledge the current study is the first study to assess both the clinical outcome and *in-vitro* microbiological response of bacterial isolates to commonly prescribed antibiotics among hospitalized patients with community acquired pneumonia in this setting. This has invaluable input for the establishment of local sensitivity protocol that leads to evidence based patient management. It is also help full as baseline study for further large scale studies in the future. But the current study is not without limitation. The follow up of patients was limited to the hospital stay of the study participants. Moreover, we were unable to identify and test *in-vitro* microbiological response of all potential bacterial cause of pneumonia and assess clinical response for options of antibiotics used for empirical therapy of community acquired pneumonia. Prospective study which could substantiate the findings of the current is needed to be done.

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CONFLICT OF INTEREST

All authors have no conflict of interest related to the submitted work.

AUTHOR CONTRIBUTIONS

G. B. devised the project, obtained funding, analyzed the data,interpreted, drafted and revised the manuscript. S. S. helped develop the methods, undertook the analyses and contributed to drafting and revision of the manuscript. S. F. and K.A. helped devise the project and methods, co-supervised the project, and helped revise the manuscript.

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