Antimicrobial susceptibility pattern of bacterial isolates from community-acquired pneumonia patients in Jimma University Specialized Hospital, Jimma, Ethiopia

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ABSTRACT

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Background: Knowing etiology and antimicrobial susceptibility patterns of bacterial isolates from patients with community-acquired pneumonia (CAP) is important in reducing the morbidity and mortality. Objective: To determine the bacterial etiologies and antimicrobial susceptibility pattern of isolates from patients with CAP. Materials and Methods: Cross-sectional study was conducted from March to July 2012. Sputum and blood specimens were collected, and microbiological investigations were performed using standard procedures. Data were presented using descriptive statistics. Results: Bacterial isolates from both sputum and blood cultures of patients were similar. Most common bacterial isolates from gram-positive bacteria were Streptococcus pneumoniae (12.8%) and Staphylococcus aureus (10.5%) and from gram-negative bacteria were, Pseudomonas aeruginosa (6.8%), Klebsiella. pneumoniae (5.3%), and Escherichia coli 3.8%. Most S. pneumoniae isolates were resistant to oxacillin (55%). High resistance rates of S. aureus isolates were observed to tetracycline (100%), penicillin (81.3%), trimethoprim-sulfamethoxazole (81.3%), erythromycin (75%), and doxycycline (50%). Gram-negative bacteria isolates were resistant to tetracycline (66.7-100%), doxycycline (50-100%), trimethoprim-sulfamethoxazole (66.7-100%), and ampicillin (66.7-100%). Resistance to two or more drugs was also observed among 62.7% of bacterial isolates, Conclusion: High rate of antimicrobial resistance was observed to commonly prescribed antimicrobial agents for CAP as empiric therapy.

Key words: Antimicrobial susceptibility pattern, bacterial etiology, community-acquired pneumonia

INTRODUCTION

Community-acquired pneumonia (CAP) continues to be one of the leading causes of hospitalization and death among patients in most developing countries.^[1,2]

The 2004 World Health Organization Global Burden of Disease Study estimated that lower respiratory tract infections (LRTIs), which include CAP, were responsible for 429.2 million episodes of illness worldwide and also were the leading cause of disease burden measured in terms of disability-adjusted life

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years (DALYs). In adults aged over 59 years, 1.6 million deaths annually are attributed to CAP. The burden of CAP is even greater concern for aging adults when considering that the number of persons aged over 60 years globally is projected to triple, from 759 million in 2010 to 2 billion by 2050.^[3]

Previous studies conducted in different parts of the world indicated that the leading bacterial causative agents of CAP are *Streptococcus pneumonia (S. pneumoniae)* and *Haemophilus influenza (H. influenza)* followed by *Staphylococcus aureus (S. aureus)*.^[2-7]

A study conducted in Ethiopia at Addis Ababa's Black Lion Hospital, on adult patients with CAP revealed that S. *pneumoniae* (6%) and S. *aureus* (6%) were the most common pathogens followed by *Pseudomonas aeruginosa* (*P. aeruginosa*) (1%), and *Klebsiella pneumonia* (K. *pneumoniae*) (1%).^[5]

The selection of empirical therapy for CAP has become complicated by the rapid development of drug resistance by common bacterial causative pathogens of CAP toward commonly prescribed drugs. *S. pneumonia* and *H. influenzae* (the common causative agent of CAP) reportedly showed resistance to penicillin, macrolides, chloramphenicol, and cotrimoxazole. The resistant strains of bacteria can quickly multiply and spread within the community.^[8-10]

Isolation, identification, and susceptibility testing to guide treatment are not feasible for each individual patient because of the time required for the laboratory procedures and the cost for limited resource countries like Ethiopia. Treatment is therefore usually empirical. Empirical treatment should be based on the knowledge of most likely etiological agent and local drug susceptibility pattern of the most likely pathogens. Thus, the current study presented data on bacterial etiologies and antibacterial susceptibility of the isolated bacteria from CAP patients.

MATERIALS AND METHODS

Study period and subjects

This facility-based cross-sectional study was conducted from March to July 2012. All adults of age > 18 years with typical symptoms of the disease (cough, fever, and/or chest pain) and the presence of opacity on the chest radiograph consistent with pneumonia and who consented to participate in the study were included. However, those patients who were under antibiotic treatment during the time of specimen collection as well as patients who were admitted for more than 48 hours were excluded from the study.

Ethical clearance was secured from Ethical Clearance Committee of Jimma University College of Public Health and Medical Sciences. Permission was obtained from the Medical director of University Specialized Hospital. Written informed consent was obtained from each patient participating in the study. Any data pertaining to the patient were kept confidential.

Demographic, laboratory, and clinical data

All relevant demographic, clinical, and laboratory data were collected through face-to-face interviews with the patient and structured questionnaire were used to collect data. Physical examination was conducted by the physician and chest x-ray comment of the radiologist from the patient card was considered.

Microbiological procedures

Sputum from all patients and blood specimens from febrile (>37.8°C) patients with CAP were collected and brought to the Medical Microbiology Laboratory of Jimma University for laboratory processing/investigation.

Sputum: Specimens were first inspected both macroscopically and under a microscope after gram staining to judge about the source specimen whether it is from lower respiratory tract

or not. The specimens were accepted for further culturing if they contained at least 25 polymorphonuclear leukocytes per low-power field.^[4,11] Sputa was liquefied with dithiothreitol and diluted at a 1:1000 ratio with sterile distilled water, and inoculated onto blood agar, MacConkey agar, manitol salt agar (MSA; Oxoid Ltd, Basingstoke Hampshire, UK), and Chocolate agar plates. The plates were incubated aerobically, except for chocolate agar, which was incubated in 5-10% CO2 concentrated candle jar, at 35°C for 24 hours.^[12]

Blood: About 10 ml of venous blood was drawn aseptically from each patient by cleaning the skin using tincture of iodine, and inoculated into brain heart infusion (BHI) broth (Oxoid Ltd, Basingstoke Hampshire, UK) containing 0.05% sodium polyanetholesulfonate (Oxoid Ltd, Basingstoke Hampshire, UK). A minimum blood-to-broth ratio of 1 in 10 was maintained.^[13] Blood culture broths were incubated at 37°C and checked for signs of bacterial growth daily for up to 7 days. Bottles that showed signs of growth were sub-cultured onto blood agar, chocolate agar, MSA, and MacConkey agar plates. Blood culture broth with no bacterial growth after 7 days were sub-cultured before being reported as a negative result; all plates were incubated as mentioned above and presumptive identification was made with morphology and hemolytic activities of colonies.^[14,15]

Identification of bacteria

On blood agar *S. pneumoniae* were differentiated from other alpha hemolytic streptococci by using an optochin (ethylhydroxycupreine) disk; *S. pneumoniae* is inhibited by optochin. *S. pneumoniae* isolates were also confirmed by the bile solubility test. X and V factors required for growth of H. *influenzae* were used to confirm colonies of this bacterium sub-cultured from chocolate agar plates. Isolates were identified as *S. aureus* by their growth on MSA, colonial morphology, hemolytic activity on blood agar plate, and their catalase as well as coagulase positive test results after sub-culturing to nutrient agar.^[16]

Bacteria that grow on MacConkey agar plate were inoculated on nutrient broth and different biochemical media such as Motility Indole Urea (MIU), Lysine decarboxylase (LDC), oxidase, KIA/TSI and citrate utilization tests were used to identify different gram-negative bacteria that could be associated with CAP.^[17] Gram stain was done whenever it was deemed necessary.

Susceptibility testing: Antimicrobial drug susceptibility testing was carried out using disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines.^[17,18] The antibiotic discs used and their concentrations were: Ceftriaxone (CRO, 30 μ g), ciprofloxacin (CIP, 5 μ g), tetracycline (TE, 30 μ g), chloramphenicol (C, 30 μ g), erythromycin (E, 15 μ g), doxycycline (DO, 30 μ g), penicillin (P, 10 IU), gentamycin (CN, 10 μ g), trimethoprim-sulfamethoxazole (TMP-SMX, 1.25 + 23.75 μ g), ampicillin (AMP, 10 μ g), and oxacillin (OXA, 1 μ g). In this particular study, the antimicrobial agents used for testing the

antimicrobial susceptibility of the isolates were those that are used for empiric treatment of CAP in the study area. All antibiotic were obtained from Oxoid Limited, Basingstoke Hampshire, UK. A standard inoculum adjusted to 0.5 McFarland was swabbed on to Mueller-Hinton agar (MHA, Oxoid Ltd. Bashingstore, Hampaire, UK); antibiotic disc were dispensed after drying the plate for 3–5 min and incubated at 37°C for 24 hours. For *S. pneumoniae*, MHA (Oxoid) supplied with 5% sheep blood and for *H. influenzae*, MHA chocolate agar was used. Quality control strains that were used for growth and drug potency included: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853.^[18] Multidrug resistant was defined if the bacterial isolate is resistance to two or more drugs tested.

Data management and analysis

Data were collected and checked for completeness, edited, cleaned and entered and analyzed using SPSS version 16.0 computer software. The data were expressed using descriptive statistics and percentages.

RESULTS

Socio-demographic characteristics and clinical features

During the study period from March to July 2012, total 133 adult patients clinically diagnosed to have CAP at Jimma University Specialized Hospital (JUSH) were selected and participated in this study. Of these, 79 (59.4%) were males and 54 (40.6%) were females showing overall male to female ratio 1.5:1. The minimum and maximum age of the patient were18 and 65 years, respectively with mean age of patients was 34.4 years (+1.56 *SD*).

Etiologic agents

Sputum culture was performed for all 133 adult patients of whom 60 (45%) of the samples were positive for bacterial growth. *S. pneumonia* 17 (12.8%), *S. aureus* 14 (10.5%), and *P. aeruginosa* 9 (6.8%), were the three common bacteria isolated in the order of frequency [Figure 1].



Figure 1: Frequency of bacterial isolates from sputum specimens of adult patients with community acquired pneumonia in Jimma University specialized hospital, 2012

Blood culture was performed for 50 CAP adult patients who were febrile during the time of data collection and overall positive culture yield was 7 (14%); of these *Streptococcus pneumoniae*, 3 (6%) were the commonest followed by *S. aureus* 2 (4%), *Pseudomonas aeruginosa* 1 (2%), and *Klebsiella pneumoniae* 1 (2%).

Mixed infection was demonstrated in 6 (4.5%) adult patients. Both *Proteus mirabilis (P. mirabilis)* and *S. aureus* were isolated from 2 (1.5%) patients, while *K. pneumoniae* and *E. coli*, *S. aureus*, and *P. aeruginosa; K. pneumoniae* and *P. mirabilis;* and *S. aureus* and *H. influenzae* were isolated from one patient (0.8%) each.

Antimicrobial susceptibility

Among 20 S. pneumoniae isolates, overall resistance rate was high to oxacillin 11 (55%) and low resistance were observed for tetracycline 7 (35%), erythromycin 1 (5%), and chloramphenicol 1 (5%); while none of them were resistant to trimethoprim-sulfamethoxazole. Of 16 S. aureus isolates subjected to antimicrobial susceptibility testing, overall resistance rate were high to tetracycline 16 (100%), oxacillin 13 (81.3%), ampicillin 13 (81.3%), penicillin 13 (81.3%), trimethoprim-sulfamethoxazole 13 (81.3%), erythromycin 12 (75%), and doxycycline 8 (50%). Low resistance were observed for ceftriaxone 5 (31.3%), ciprofloxacin 5 (31.3%), chloramphenicol 5 (31.3%), and gentamycin 5 (31.5%). *P. aeruginosa* isolates showed relatively high resistance to gentamycin 5 (50%). Low resistances were observed for ceftriaxone 2 (20%) and ciprofloxacin 2 (20%). The antimicrobial testing of K. pneumonia, P. mirabilis, P. vulgaris, E. coli, and H. influenza isolates showed that all (100%) isolates had high resistance to tetracycline, ampicillin, and trimethoprim-sulfamethoxazole. But low or no resistance was observed to ciprofloxacin and ceftriaxone [Table 1].

Antibiogram of bacterial isolates showed that all isolates were resistant to two or more drugs except *S. pneumonia* and *H. influenza*, which showed only 15% and 66.7% of the isolates were resistant to two or more drugs, respectively [Table 2].

DISCUSSION

In this study, bacterial pathogens were isolated in 45% of the sputum specimens. The pathogen isolation data in this study indicated that *S. pneumoniae* was the most prevalent (12.8%) isolate, followed by *S. aureus* (10.8%), gram-negative bacilli (19.5%) including *P. aeruginosa* (6.8%), *K. pneumoniae* (5.3%), *E. coli* (4.5%), *Proteus* species (3.1%), and *H. influenza* (2.3%). Comparable isolation pattern have been reported in the surveys carried out in Nigeria,^[1,19] Singapore,^[2] Chile,^[15] Iran,^[20] India,^[21] Nicaragua,^[22] and Ethiopia.^[5] This may point out that the variability in the pattern of etiologic agent for CAP is minimal.

Incidence of mixed bacterial infection in this study was 4.5%. It is comparable with a study conducted in Nigeria (4.7%).^[23]

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Table 1: Drug resistance pattern of bacterial isolates from adult patients with Community acquired pneumonia in Jimma **University Specialized Hospital, 2012**

Bacterial	Drugs tested No (%) resistance											
isolates	No	CRO	CIP	TE	С	Е	DO	Р	CN	TMP-STX	AMP	ΟΧΑ
S. pneumoniae	20	NA	NA	7 (35)	1 (5)	1 (5)	NA	NA	NA	0 (0)	NA	11 (55)
S. aureus	16	5 (31.3)	5 (31.5)	16 (100)	5 (31.5)	12 (75)	8 (50)	13 (81.3)	5 (31.5)	13 (81.3)	13 (81.3)	13 (81.3)
P. aeruginosa	10	2	2	NA	NA	NA	NA	NA	5	NA	NA	NA
K. pneumoniae	8	2	0	8	2	NA	0	NA	2	8	8	NA
P. mirabilis	4	1	1	4	4	NA	4	NA	4	4	4	NA
P. vulgaris	1	0	0	1	1	NA	1	NA	0	1	1	NA
E. coli	5	0	0	5	5	NA	5	NA	2	5	5	NA
H. influenzae	3	1	1	2	1	NA	NA	NA	NA	2	2	NA

NA: Note applicable, CRO: Ceftriaxone, CIP: Ciprofloxacin, TE: Tetracycline, C: Chloramphenicol, E-Erythromycin, DO: Doxycycline, P: Penicillin, CN: Gentamycin, TMP-STX: Trimethoprim-sulfamethoxazole, AMP: Ampicillin, and OXA: Oxacillin

Table 2: Multi-drug resistance antibiogram of bacterial isolates from adult patients with community-acquired pneumonia in Jimma University Specialized Hospital, 2012

Bacterial isolates	Resistance antibiogram	No (%)
S. pneumoniae	OXA, TE	2 (10)
(<i>n</i> =20)	OXA, TE, C, E	1 (5)
<i>S. aureus</i> (<i>n</i> =16)	OXA, TE, P, AMP	2 (12.5)
	OXA, AMP, E, DO, TMP-STX	1 (6.3)
	P, TE, E, DO, TMP-STX	1 (6.3)
	OXA, AMP, TE, E, DO, TMP-STX	2 (12.5)
	OXA, AMP, P, TE, E, TMP-STX	1 (6.3)
	OXA, AMP, P, TE, DO, TMP-STX	1 (6.3)
	OXA, AMP, P, TE, DO, E, TMP-STX	1 (6.3)
	OXA, AMP, P, TE, DO, C, E, CIP, TMP-STX	1 (6.3)
	OXA, AMP, P, TE, C, E, CN, CRO, TMP-STX	<mark>2 (12.5</mark>)
	OXA, AMP, P, TE, C, E, CN, CRO, CIP, TMP-STX	4 (25)
P. aeruginosa	CN, CRO	2
(<i>n</i> =10)	CN, CRO, CIP	1
K. pneumoniae	AMP, TE, TMP-STX	3
(<i>n</i> =8)	AMP, TE, CRO, TMP-STX	2
	AMP, TE, CN, TMP-STX	1
	AMP, TE, C, TMP-STX	1
	AMP, TE, CN, CRO, TMP-STX	1
P. mirabilis (n=4)	AMP, TE, DO, C, CN, TMP-STX	3
	AMP, TE, DO, C, CN, CRO, CIP, TMP-STX	1
P. vulgaris (n=1)	AMP, TE, DO, C, TMP-STX	1
<i>E. coli</i> (<i>n</i> =5)	AMP, TE, DO, C, TMP-STX	3
	AMP, TE, DO, C, CN, TMP-STX	2
H. influenzae	AMP, TE, TMP-STX	1
(<i>n</i> =3)	AMP, TE, C, CIP, TMP-STX	1
Total (<i>n</i> =67)		42 (62.7)

NA: Note applicable, CRO: Ceftriaxone, CIP: Ciprofloxacin, TE: Tetracycline, C: Chloramphenicol, E: Erythromycin, DO: Doxycycline, P: Penicillin, CN: Gentamycin, TMP-STX: Trimethoprim-sulfamethoxazole, AMP: Ampicillin, and OXA: Oxacillin

It is also consistent with the fact that the incidence of mixed infections does not usually exceed 30% as has been observed in another study.^[24] However, the identification of polymicrobial infection is very important for treatment strategies and to avoid a false impression of clinically resistant strains.

S. pneumoniae, which was the most common isolate in this study, showed 55% resistance to oxacillin, which was comparable with studies conducted in Iran^[22] where 30-57% of the isolates were resistant. All S. pneumoniae isolates from this study were susceptible to trimethoprim-sulfamethoxazole. In contrast to this, studies conducted in Nigeria^[23] all isolates and in Kenya^[25] 54% of the isolates were indicated as resistant to trimethoprim-sulfamethoxazole. On the other hand, more than 95% of tested S. pneumoniae isolates were susceptible to chloramphenicol and erythromycin, which is comparable with a study conducted in Kenya where >97% isolates were resistant to the mentioned antibiotics.^[25]

In this study, 68.7% S. aureus showed susceptibility to ceftriaxone, ciprofloxacin, gentamycin, and chloramphenicol and was comparable with study results conducted in Ibadan, Nigeria where 83.3%, 66.7%, 66.7%, and 83.3% of the isolates were susceptible to those mentioned drugs, respectively^[26] as well as with study findings reported from Benin, Nigeria where 66.7%, 66.7%, 80%, and 66.7% of the isolates were susceptible to similar drugs.^[27] Most (85.7%) of the S. aureus showed resistance to penicillin, ampicillin, oxacillin, and trimethoprim-sulfamethoxazole; comparable result was also reported from China (88.7% resistance to Penicillin)^[26] and Nigeria (66.7% resistant to penicillin and 100% for trimethoprim-sulfamethoxazole).^[23]

In our study, 70-100% gram-negative bacilli isolates were sensitive to ceftriaxone and ciprofloxacin. These findings are comparable with studies conducted in Benin City, Nigeria^[28] and Ibadan, Nigeria^[23] where 66-100% of the isolates were susceptible to those mentioned drugs. Except K. Pneumoniae, all isolates of gram-negative bacilli were resistant (100%) to tetracycline, chloramphenicol, doxycycline (susceptible), trimethoprim-sulfamethoxazole, and ampicillin, and comparable result was reported from Nigeria^[27] where 60-100% of the isolates were developed resistance to the mentioned drugs.

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The most common causative agent among gram-negative bacilli, *P. aeruginosa*, showed 50% resistance to gentamycin, which is comparable with the study conducted in Nigeria (with resistant rate of 53.6%).^[26] However, it showed low resistance (20%) to ceftriaxone and ciprofloxacin. Similarly study conducted in Nigeria showed 39.3% resistance for ciprofloxacin and no resistance for ceftriaxone.^[23] *K. pneumoniae* and *E. coli* showed 100% resistance to tetracycline and trimethoprim-sulfamethoxazole, which was parallel with studies conducted in Benin City, Nigeria where all isolates were resistant to tetracycline)^[27] and Ibadan, Nigeria with 100% resistance to trimethoprim-sulfamethoxazole.^[23] In line with the study conducted in Nigeria all *Proteus* species were also resistant to trimethoprim-sulfamethoxazole.^[26]

The limitation of our study is that we did not attempt to isolate and identify some common pathogens like *Chlamydia*, *Mycoplasma*, *Legionella* species, etc., and serotyping was not done to *H. influenzae* due to resource limitation. Some patients who were given antibiotics before/on admission to hospital were included in the study.

All *H. influenzae* isolates in this study showed low resistance (33.3%) to ceftriaxone, ciprofloxacin, and chloramphenicol, which was parallel to study findings in Nigeria where 13-30.3% isolates were resistant to the same drugs.^[23] High resistance rate (66.6%) to tetracycline, ampicillin, and trimethoprim-sulfamethoxazole were observed among *H. influenzae* isolates, which is comparable with study conducted in USA (47% resistance to ampicillin)^[28] and Nigeria (93.7% resistance to Trimethoprim-sulfamethoxazole)^[26] but is not as high as that observed in other countries such as in China (>90% susceptibility to most antibiotics).^[29] These differences could be due to variations in antibiotic prescribing habits among the two countries.

The antibiogram result showed that all (100%) *S. aureus* and gram-negative bacilli (except *P. aeruginosa*) isolate showed multidrug resistant (MDR) to the commonly prescribed drugs. *S. pneumoniae*, *S. aureus*, from gram-positive; *P. aeruginosa*, *K. pneumoniae*, *proteus spps*, and *E. coli* from gram-negative were the commonly isolated strains in this study. It seems the types of bacteria responsible for CAP are similar to studies conducted elsewhere regardless of differences in different factors. However, the frequency of occurrence and the susceptibility pattern of the isolates are different.

However, based on our finding all isolates showed multiple drug resistance to these commonly used antimicrobial agents. Only few drugs like ceftriaxone and ciprofloxacin were found effective. If used more and more without control, these isolates may also develop resistance to these drugs too. For now and the future, the empiric treatment of CAP may be challenging. This is mainly drugs that are fairly available and frequently used were ineffective (based on *in vitro* susceptibility test). Alternative management modalities should be sought, especially in developing countries where introduction of new brand drugs is economically challenging and the disease is also prevalent and severe.

In our study, most strains isolated were resistant to frequently prescribed antimicrobials in the particular hospital. This could be due to widespread misuse of antimicrobials by the patient due to the lack of access to appropriate treatment and under use due to inadequate dosing, poor drug quality, and incomplete treatment courses or through inappropriate prescribing habits and an over-zealous desire to treat every infection, which should be further explored.

Provided that empiric therapy is unavoidable in management of CAP; the efficacy of antimicrobials at hand is important. However, in developing countries like Ethiopia, the available drugs are limited (limited options of antimicrobials) enforcing use of similar antimicrobials frequently. These, in turn, in the absence of sufficient and appropriate guiding studies may elaborate the problem. As a result, the problem might have been under scored. We believe the antibiotic resistance observed is higher than has been demonstrated in this study.

In Ethiopia, the unregulated over-the-counter sale of these antimicrobials, mainly for self-treatment of suspected infection in humans without prescription, would inevitably lead to emergence and rapid dissemination of resistance. In addition, availability of cheaper generic drugs of variable quality in the market for treatment of bacterial infections may also contribute to the increased level of resistance. A study on practice of self-medication in Jimma town showed that 27.6% were reported self-medicated. The relative lesser cost (35.7%) was the major reason for using self-medication.^[30]

We believe that this is unnoticed challenge in the clinical scenario and intervention strategies directed at establishment of antibiotic policy, education of prescribers and community, establishment of a novel prescription system will reduce the inappropriate use of antibiotics. Hence, further studies should be conducted in a broader context to guide effective and/or alternative tailor-made treatment guidelines.

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