Journal of Medical Laboratory and Diagnosis Vol. 2(3), pp. 27-30, July 2011
Available online http://www.academicjournals.org/JMLD
ISSN 2141-2618 ©2011 Academic Journals

Full Length Research Paper

Streptococcus pneumonia and antimicrobial resistance, Hawassa Referral Hospital, South Ethiopia

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Accepted 20 April, 2011

Bacterial resistance to antimicrobial agents is an increasing problem in many areas of the tropics. To assess drug resistance pattern of *Streptococcus pneumoniae* among clinically diagnosed cases of pneumonia, meningitis and otitis media in Hawassa Referral Hospital, Southern Ethiopia. A cross-sectional survey was conducted on 152 cases from sputum, cerebrospinal-fluid, and ear discharge samples. Blood agar and Muller-Hinton agar were used to culture samples. Biochemical tests and antibiotic susceptibility tests were also done. Gram staining and microscopic examinations were carried out. The mean age of the study subjects was 26.9 years (range: 1 month to 55 years); 61.2% were males. Of cultured 152 patients' samples 21.4% growth *Streptococcus pneumoniae*. The highest resistance rate was seen for ampicillin and penicillin but lowest for chloramphenicol. Sixty four point two percent (64.2%) of the isolates were resistant to two or more antimicrobial agents. *S. pneumoniae* shows highest resistance for ampicillin and penicillin.

Key words: *Streptococcus pneumoniae,* drug resistance, antimicrobial agents, antibiotics susceptibility test.

INTRODUCTION

The pneumococcus (*Streptococcus pneumoniae*) continues to be a common cause of serious and life-threatening infections, including pneumonia, bacteraemia and meningitis. It is also a frequent cause of respiratory tract infections, such as otitis media and sinusitis (Klugman, 1990; Collignon, 1992). The *S. pneumoniae* continues to cause significant illness worldwide, and with challenging antibiotic resistance issues (Lister, 1995; Schreiber and Jacobs, 1995). *S. pneumoniae* is a leading cause of morbidity and mortality in the United States, resulting each year in an estimated 3,000 cases of meningitis, 50,000 cases of bacteremia, 500,000 cases of pneumonia, and 7,000,000 cases of otitis media (Reichler et al., 1992). During the first half of the 20th century *S. pneumonia* was susceptible to penicillin and most other antimicrobial agents. However, in recent years *S. pneumonia* has exhibited increased resistance to standard agents including penicillin, erythromycin, chloramphenicol, and extended spectrum cephalosporins (Collignon and Turnidge, 2000).

Since antibiotics first became widely used in the World War II era, they have saved countless lives and blunted serious complication of many feared infectious diseases, however many antimicrobials are not as effective as they used to be. Widespread use of antibiotics is thought to have spurred evolutionary adaptations that enable bacteria to survive these powerful drugs. For many years *S. pneumonia* was uniformly susceptible to penicillin G with MICs of <0.1 µg/ml. In the past, *S. pneumoniae* was almost uniformly susceptible to penicillin, allowing most physicians to treat persons who had severe infections with penicillin alone without testing for resistance. Since the 1960s, however, resistance to penicillin and other antimicrobial agents has spread rapidly and was first reported in Australia in 1967, in New Guinea in 1969, in

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South Africa in 1977, and in many other countries throughout Africa, Asia, and Europe (Caputo et al., 1993; Jacobs et al., 1978). Penicillin resistance and multi-drug resistance have spread worldwide (Collignon and Bell, 1992). Many penicillin resistant strains are also resistant to other drugs such as chloramphenicol, erythromycin, tetracycline and trimethoprim-sulfamethoxazole (Moreillon et al., 2000).

As multi-drug resistant strains become increasingly prevalent, treatment options will become limited. The clinical impact of antimicrobial resistances on the outcomes of invasive and non- invasive DRSP infections remains largely unknown. To make appropriate empiric antimicrobial choices clinicians need a reliable and current assessment of the level of antimicrobial resistance in the community (Kentuck, 1994; Gonzales et al., 2001). Surveillance data collected at the center for disease control and prevention (CDC) have shown that high level resistance to penicillin increased more than 60-fold from 0.02% for 1979 to 1987 to 1-3% in 1992 for pneumococcal isolates from invasive infections (Breiman et al., 1994). In some communities, at least 30% of isolates are non-susceptible to penicillin (Whitney et al., 2000).

The purpose of this study is to observe the recent prevalence of pathogenic drug resistant strains of *S. pneumonia*; among clinically diagnosed cases of pneumonia in Hawassa Referral Hospital and to enhance proper use of antibiotics to provide up- to-date information for health care workers.

METHOD

A cross-sectional study was conducted in Hawassa Referral Hospital, Southern Ethiopia, to assess the prevalence of *S. pneumonia* drug resistance on samples collected from patients who attended the hospital as in-patient and outpatient. All patients who come to Hawassa Referral Hospital for medical care during study period were taken as the source population. A total of 152 patients with clinically diagnosed pneumonia, otitis media or meningitis were included in the study from July 1st, 2008 to January 31st, 2009. Patient's sputum, CSF or ear discharge samples were collected using standard method for culture and gram staining. Subjects were also provided with questionnaire to identify risk factors for drug resistances *S. pneumonia* (DRSP) acquisition which includes previous hospitalization, respiratory disease, intravenous drug use, use of antibiotics for any case within the past year, chronic illness and TB treatment.

A sample collected from a suspected patient was cultured directly on the media within 4 h of sampling; plates were then incubated at 35 to 37°C for 24 to 48 h and for limited time at room temperature to stimulate pigment formation. The media used for culture were blood agar and Muller-Hinton agar. Individual colonies were tested for the production of pigments and other characteristic by biochemical tests including bile solubility test and optochin sensitivity test. Susceptibility testing was carried out by using modified Kirby-Bauer agar disc diffusion method with antibiotic discs of the same brand (Bauer et al., 1966). Gram stains and microscopic examinations were done for all obtained samples. Isolates were tested for susceptibility to erythromycin (15 µg), chloramphenicol (30 µg), ampicillin (10 µg), penicillin (10 µg), ceftriaxone (10 µg) and cotrimoxazole (10 µg) by blood agar disc diffusion technique. Those colonies of *S. pneumonia* and control organisms which are resistant to a drug were taken as DRSP. Format was prepared to fill results from culture and susceptibility results. The final data file was compiled and imported into SPSS version 16.3 computer database for analysis. Recoding and re-categorizing was made for relevant variables as needed. Analysis consisted of basic summaries of patients' characteristics using tables and figures.

Quality control methods includes: 1) using standardized sterile cotton swabs of culture transport system [Difco, Detroit], 2) use of quality culture Medias of Difco, 3) use of modified Kirby-Bauer agar disc diffusion testing procedure for susceptibility testing and monitoring of specimens collection, 4) processing and analysis of the data by the investigators. The research was conducted after getting ethical clearance from the Hawassa University College of Health Sciences Institutional Review Board Committee (IRB). Consent form was prepared in English and Amharic version. Informed consent was obtained from all study participants.

RESULTS

The mean age of participants was 26.9 years range from one month to 55 years. 93 (61.2%) were males. A total of 152 samples from different patients were tested. Out of the cultured 152 sputum, cerebrospinal fluid and ear discharge samples 145 (95.4%) showed a growth of microorganism: 31 (21.4%) showed *S. pneumonia* and 114 (78.6%) showed non *S. pneumonia*. Optochin sensitive *S. pneumonia* isolates were 4 from CSF, 23 from sputum and 4 from ear discharge samples (Table 1). The Kirby-Bauer sensitivity test for the 31 isolates of *S. pneumonia* against six commonly used antibiotics is shown in Table 2.

The highest resistance rate 29 (93.5%) was for ampicillin and lowest for chloramphenicol 1 (3.2%). All six antibiotics were tested on 31 isolates and 20 (64.2%) of the isolates were resistant to two or more antimicrobial agents (Table 3). Ampicillin resistance alone was seen in 11 isolates and the remaining and the remaining 20 isolates showed ampicillin resistance along with resistance to one or more antibiotics. Only three of the 31 isolates had intermediate resistance to cotrimoxazole and erythromycin. Sex, age, prior antimicrobial therapy for the current problem or within the last 6 months, setup (admission or outpatient) and underlining condition like TB did not predict or explain the resistance pattern significantly.

DISCUSSION

Several studies have confirmed that *S. pneumonia* is an important cause for pneumonia, meningitis and ottits media (Klugman, 1990; Collignon, 1992; Zinser, 1980). Although, antimicrobials were of help in the initial phase of their development, the fast emergence of drug resistant *S. pneumonia* strains have created a problem in the control and treatment of various infections (Feikin et al.,

Table 1. Number of *S. pneumonia* and other non *S. pneumonia* growth using blood agar and chocolate agar among 152 patients in Hawassa Referral Hospital, Ethiopia, from July 1st to January 31st, 2009.

Sample	Growth seen		No growth seen	Total
	S. pneumonia	Non *S. pneumonia*		
	No.	No.	No.	No. (%)
CSF*	4	1	6	11(7.2)
Ear discharge	4	9	1	14(9.2)
Sputum	23	104	0	127(83.6)
Total	31(21.4)	114(78.6)	7(4.6)	152(100)

CSF= cerebrospinal fluid.

Table 2. Antibiotic sensitivity of 31 *S. pneumonia* isolates from CSF, sputum and ear discharge in Hawassa Referral Hospital, Ethiopia, from July 1st to January 31st, 2009.

Antibiotic	*Streptococcus pneumonia* (n=31)		
	Resistance	Intermediately sensitive	Sensitive
	No. (%)	No. (%)	No. (%)
Ampicillin, 25 µg	29(93.5)	0(0.0)	2(6.5)
Penicillin, 10 µg	20(64.5)	0(0.0)	11(35.5)
Ceftriaxon, 30 µg	9(29.0)	0(0.0)	22(71.0)
Erythromycin, 15 µg	4(12.9)	1(3.2)	26(83.9)
Cotrimoxazole, 25 µg	0(0.0)	2(6.5)	29(93.5)
Chloramphenicol, 30 µg	1(3.2)	0(0.0)	30(96.8)

Table 3. Antibiograms of 31 *S. pneumonia* isolates of different samples in Hawassa Referral Hospital, Ethiopia, from July 1st – January 31st, 2009.

Resistance pattern	Resistant strains No. (%)
Amp	11(34.5)
Amp, pen	12(38.7)
Amp, Ceft	1(3.2)
Amp, Pen, Ceft	5(16)
Pen, ceft, Ery	1(3.2)
Amp, pen, ceft, Eryt	1(3.2)
Total	31(100)

Amp-ampicillin, Pen-Penicillin, Ceft-Ceftriaxon, Ery-Erythromycin.

2000; Breiman et al., 1994; Kentuck, 1994; Collignon and Bell, 1992). Thirty one (21.4%) *S. pneumonia* was isolated from 152 samples being tested in Hawassa Referral and Teaching Hospital within 6 months. This is smaller than the range of isolation of *S. pneumonia* in St. Christopher's hospital for children USA, 498 isolates within seven years (Deboran et al., 1999) and greater than the range of isolation of *S. pneumonia* in Australia in 27 hospitals and private laboratories within 2 years (Peter and Jan, 1996).

In this study, the level of ampicillin resistance among *S. pneumonia* isolates was higher than the other antibiotic followed by penicillin. All penicillin resistant strains were also resistant to other antibiotics such as CAF, erythromycin, ampicillin, ceftriaxone. These results were consistent with findings in other studies (Moreillon et al., 2000; Breiman et al., 1994). Penicillin resistance rates are very high in developing countries, and in some areas of Western Europe and the USA (Whitney et al., 2000). However, the rate of raise in resistance in this study also appears very similar. A recent United States study found that 25% of invasive *S. pneumonia* isolates were penicillin resistant (Hofmann et al., 1995). The finding of high level of antibiotics resistance among

S. pneumonia isolates in this study is of particular concern; in meningitis (CSF) followed by otits media (ear discharge) and pneumonia (sputum). The explanation for not having statistically significant association between resistance pattern and the possible explanatory variables such as sex, age, prior antimicrobial therapy for the current problem or within the last 6 months, setup (admission or outpatient) and underlining conditions like TB could be due to the small sample size we had.

Our study has limitation which is in relation to the small sample size we dealt with and shorter study period. In addition, isolation of *S. pneumonia* was merely by conventional biochemical tests (bile solubility test, optochin sensitivity test and colony characteristics) and does not use molecular tests like PCR or DNA finger printing. Cognizant of this limitation, our findings questioned the use of ampicillin and penicillin to treat cases caused by *S. pneumonia*. Therefore, we recommend further study with a prolonged study period or using as many health service delivery points as possible to get enough sample size that could yield statistically sound results. In addition to the study on resistance pattern, the dominant causes of pneumonia should be ascertained to help in identifying the appropriate drug of choice in the management of pneumonia in our locality.

ACKNOWLEDGEMENTS

We thank Hawassa University Research and Extension Office for the financial support with collaboration of NPT project. We also express our gratitude to Hawassa Referral Hospital staff for facilitating the data collection.

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