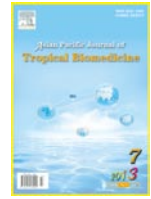




Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(13)60109-4 © 2013 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

## Antimicrobial susceptibility patterns of *Streptococcus pneumoniae* over 6 years at Gondar University Hospital, Northwest Ethiopia

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## PEER REVIEW

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## Comments

The authors have made an attempt to assess the magnitude and antimicrobial susceptibility patterns of *S. pneumoniae* isolates from various clinical specimens. The manuscript is well written and provides significant contribution and importance in the concerned area.

Details on Page 540

## ABSTRACT

**Objective:** To assess the magnitude and antimicrobial susceptibility patterns of *Streptococcus pneumoniae* isolates from various clinical specimens. **Methods:** A record based on retrospective study was conducted at Gondar University Teaching Hospital from September 2007 to January 2012. All patients who visited Gondar University Hospital and provided clinical specimens (body fluids, discharge, swab and blood) for routine bacteriological culturing and antimicrobial susceptibility testing were taken for analysis. Clinical specimens were processed for bacterial culture according to the standard procedures. Antimicrobial susceptibility test for isolated organisms was done using agar disk diffusion method. The data were entered and analyzed using SPSS software version 16 package. **Results:** One hundred and fifty three *Streptococcus pneumoniae* were isolated from patients who visited Gondar University Teaching Hospital bacteriology laboratory for culture. Majority of the pneumococcal isolates were from inpatients [111(72.5%)], and 74(48.4%) were from body fluids. Out of the total isolates, 93(61%) were found to be resistant to at least one antibiotic used for susceptibility testing. Forty eight (43.2%) of the isolates were multi-drug resistant (resistant to two or more drugs). The resistance rate noted for both ciprofloxacin 17(11.1%) and ceftriaxone 15(9.8%) were alarming. **Conclusions:** High proportions of the isolates tend to be increasingly resistant to the commonly prescribed drugs. The recommended drug of choice like ciprofloxacin and ceftriaxone were found to be less susceptible in the study area. Based on the findings, we therefore recommend that antimicrobial agents should be inspected for acceptable activity before they are prescribed and administered empirically. Further study with a better design and survey of antimicrobial susceptibility at large scale should be performed to draw advanced information.

## KEYWORDS

*Streptococcus pneumoniae*, Antimicrobial agents, Susceptibility patterns, Gondar Ethiopia

### 1. Introduction

*Streptococcus pneumoniae* (*S. pneumoniae*) remains a

common pathogen and leading cause of morbidity and mortality[1]. Transmission of *S. pneumoniae* occurs as the result of direct person-to-person contact via respiratory

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Foundation Project: This work was supported by Research and Community Services of University of Gondar with Grant No. RPO/55/43/2008.

Article history:

Received 4 May 2013

Received in revised form 15 May, 2nd revised form 26 May, 3rd revised form 2 Jun 2013

Accepted 12 Jun 2013

Available online 28 Jul 2013

droplets and by autoinoculation in persons carrying the bacteria in their upper respiratory tract[2]. This organism continues to be common causes of mild to severe and life threatening diseases including pneumonia, bacteremia and meningitis, and it is also a frequent causes of upper respiratory tract infections like otitis media and sinusitis[3].

*S. pneumoniae* was known to be completely susceptible to penicillin and other beta-lactam antibiotics. However, since 1980s, a dramatic increase in antibiotic resistance among *S. pneumoniae* has been observed in many parts of the world[4–6].

In recent times, pneumococcal strains with high level of resistance to penicillin have emerged, and these organisms have shown resistance to other antibiotics such as tetracycline, erythromycin, chloramphenicol, sulfonamide, ciprofloxacin and clindamycin. An important increase in the prevalence of penicillin resistant *S. pneumoniae* isolates occurred in the early 1990s in USA[7–9]. Such an increase had previously been observed in other parts of the world[10]. Moreover, isolates of *S. pneumoniae* that are resistant to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, erythromycin alone or in combination were being recovered more and more often in the USA[11]. This problem has also been recognized in the Asia-Pacific region[12], Europe[13] and South Africa[12] but data from the rest of African countries including Ethiopia is scarce.

In Ethiopia, there is lack of literatures on streptococcal drug resistance. However, few previous studies conducted showed the presence of resistance to commonly prescribed antibiotics except to ciprofloxacin[14]. Thus, it is imperative to inform clinicians and other health professionals, policy makers and programers about the distribution of *S. pneumoniae* and to produce a systematized data on drug susceptibility profile of this isolates in a regular base. As a result, an optimized empirical treatment policy would be designed. Therefore, this study would provide up-to-date data information on the magnitude and pattern of antimicrobial resistance of *S. pneumoniae* to the commonly prescribed antibiotics in the settings.

## 2. Materials and methods

### 2.1. Study design, period and settings

A record based on retrospective study was conducted at Gondar University Teaching Hospital, Gondar, Ethiopia from September 2007 to January 2012. Gondar town is located 737 km away from Addis Ababa and it has a projected population of 248 784. The hospital is serving more than 5 million of people from Gondar town and the adjacent regions.

### 2.2. Sampling technique and sample size

All patients who visited Gondar University Teaching

Hospital Laboratory and gave clinical specimens for bacteriological culturing and antimicrobial susceptibility testing were included in the study. All clinical specimens of the patients who gave for bacteriological culturing and antimicrobial susceptibility testing from September 2007 to January 2012 were included in the study.

### 2.3. Data collection procedure

Data on socio-demographic variables (age and sex), types of clinical specimens, sender of clinical specimen and microbiological data were abstracted from patients' records in the microbiology laboratory registration book by using a pre-prepared data abstraction format.

### 2.4. Specimen collection and processing

Specimens for the recovery of *S. pneumoniae* from nasopharyngeal, throat and wound swabs, ear and eye discharges, cerebrospinal fluid, pleural, peritoneal and ascetic fluids, pus, urine, sputum, and blood were collected aseptically with sterile cotton plugged applicator sticks and sterile test tubes as per the routine clinical management of the patients. Specimens were immediately delivered to the bacteriology section for culture and bacteriological analysis. Specimens were cultured by the streak plate methods using wire loop into blood and chocolate agars (Oxoid Basing-stoke, UK) for cultural isolation of *S. pneumoniae* from those specimens and were incubated overnight at 37 °C. The chocolate agar plates were incubated in a candle jar so as to create a reduced oxygen tension (5%–10% additional CO<sub>2</sub> tension). The media were observed for growth for up to 48 h and plates with characteristic growth of bacterial colonies were selected. Gram positivity was checked by Gram's staining procedure. Blood agar plates with alpha hemolytic colonies were isolated and tested for Optochin sensitivity so as to differentiate *Pneumococci* from other alpha hemolytic *Streptococci*. The alpha hemolytic isolates with characteristic colonies of *S. pneumoniae* which were optochin sensitive were presumptively taken as being *S. pneumoniae*. The disk diffusion test was performed to test susceptibility of the *S. pneumoniae* following the method of Bauer *et al.* A McFarland 0.5 standardized suspension of the bacteria in 0.85% sterile saline was then prepared and swabbed over the entire surface of chocolate agar plate (Oxoid Basking-stoke, UK) with a sterile cotton swab. A ring of disks (Mast Diagnostic, UK) containing single concentration of each antimicrobial agent was then placed onto the inoculated surface. Following overnight incubation at 37 °C, clear zones produced by antimicrobial inhibition of bacterial growth were measured in millimeters using straight-line ruler. The zone of diameter was interpreted using an interpretive chart for zone sizes as sensitive, intermediate and resistant as per the standard protocol[15]. The following antimicrobial agents and concentrations were used:

penicillin (10 IU), ampicillin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), co-trimoxazole (25 µg), gentamicin (10 µg), ciprofloxacin (5 µg), and ceftriaxone (30 µg). Reference strains, *E. coli* ATCC25922 and *S. aureus* ATCC25923 were used as controls.

### 2.5. Data processing and analysis

Data were entered and analyzed using SPSS statistical software version 16 package. Descriptive statistics were used to present the results of this study.

### 2.6. Ethical consideration

The study was conducted after obtaining institutional ethical clearance from research and publication office of University of Gondar. Permission was taken from Gondar University Teaching Hospital administrators and Bacteriology section. In addition, all information that was necessary for the management and further investigation was given to the responsible health professionals to create awareness.

## 3. Results

During the 6-year period from September 2007 to January 2012, a total of 153 *S. pneumoniae* isolates had been recovered from patients who visited Gondar University Teaching Hospital bacteriology laboratory for specimens cultured. Of the 153 isolates, 29(19%) were from male children less than 5 years of age and 26(17%) were from female children less than 5 years of age (Table 1).

**Table 1**

Distribution of *S. pneumoniae* isolates with age, sex, and sender of clinical specimen at Gondar University Teaching Hospital, Northwest Ethiopia from September 2007 to January 2012 [*n*(%)].

Characteristic	Frequency			
	Male	Female	Total	
Age	≤5	29(19.0)	26(17.0)	55(36.0)
	6–15	21(13.7)	17(11.1)	38(24.8)
	16–34	23(15.0)	17(11.1)	40(26.2)
	35–55	11(7.2)	6(3.9)	17(11.1)
	>55	1(0.7)	2(1.3)	3(1.9)
Sources	OPD	18(11.8)	24(15.7)	42(27.5)
	Ward	59(38.4)	52(34.0)	111(72.5)
Total	77(50.3)	76(49.7)	153(100.0)	

*n*: individual number; OPD: Out Patient Department.

Of the 153 pneumococcal isolates, 74(48.4%) were from body fluids followed by 23(15%) from wound discharge, 19(12.4%) from eye discharge, 17(11.1%) from swabs, 8(5.2%) from ear discharge, 7(4.6%) from pus, and 4(2.6%) were from urine. Majority of the pneumococcal isolates were from ward [111(72.5%)], 59(38.4%) of which were from males and 52(34.0%) were from females (Table 2).

**Table 2**

Isolates of *S. pneumoniae* from specimens of outpatients and inpatients at Gondar University Teaching Hospital, Northwest Ethiopia from September 2007 to January 2012 [*n*(%)].

Specimens	OPD	Ward	Total
Swabs	10(6.50)	7(4.60)	17(11.10)
Body fluids	10(6.50)	64(41.80)	74(48.40)
Ear discharge	5(3.30)	3(2.00)	8(5.20)
Wound discharge	7(4.57)	16(10.50)	23(15.00)
Eye discharge	1(0.70)	6(3.90)	7(4.60)
Pus	5(3.30)	14(9.20)	19(12.40)
Urine	3(2.00)	1(0.70)	4(2.60)
Others	1(0.70)	0(0.00)	1(0.70)
Total	42(27.50)	111(72.50)	153(100)

OPD: Out Patient Department; *n*: number of isolates.

A total of 93(61%) were found to be resistant to at least one antibiotic tested. Resistance to two or more antimicrobials (multiple drug resistance) was observed in 66(43.2%) of the isolates. In detail, 80(11.8%), 13(8.5%), 8(5.2%), 13(8.5%) and 14(9.2%) of the isolates were found to be resistant to two, three, four, five, six or more antibiotics, respectively. The resistance rate noted for both ciprofloxacin 17(11.1%) and ceftriaxone 15(9.8%) were alarming. The rate of resistance for ampicillin, penicillin G, tetracycline, co-trimoxazole, chloramphenicol, erythromycin, and gentamicin were 44(29.0%), 48(31.3%), 48(31.3%), 49(32.0%), 44(29.0%), 31(20.3%), and 43(28.1%), respectively. One isolate from 1.5 year old male infant cerebrospinal fluid sample was found to be resistant to all antibiotics tested. Table 3 depicts the susceptibility pattern and the rate of MDR among the isolates.

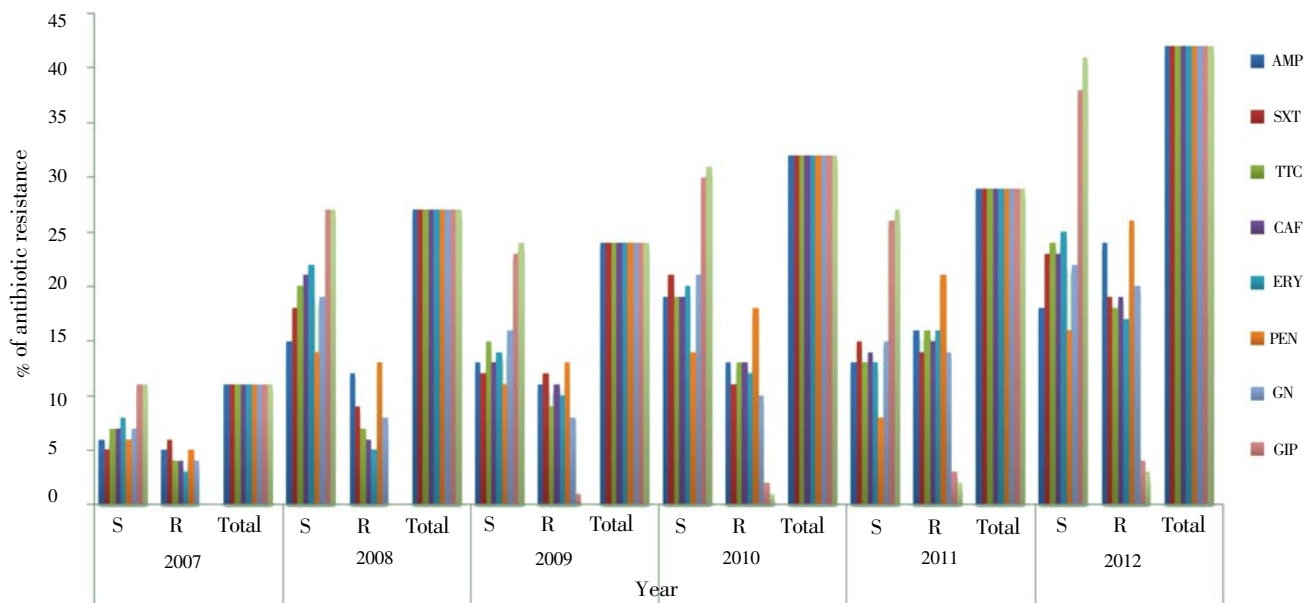
**Table 3**

*In vitro* antimicrobial susceptibility patterns of *S. pneumoniae* isolate at Gondar University Teaching Hospital, Northwest Ethiopia from September 2007 to January 2012 [*n*(%)].

Antibiotics	Susceptible isolate	Resistant (Intermediate+ Complete) isolate
Ampicillin	109(71.0)	44(29.0)
Penicillin G	105(68.7)	48(31.3)
Tetracycline	105(68.7)	48(31.3)
Co-trimoxazole	104(67.9)	49(32.0)
Chloramphenicol	109(71.0)	44(29.0)
Erythromycin	122(79.7)	31(20.3)
Gentamicin	110(71.9)	43(28.1)
Ciprofloxacin	136(88.9)	17(11.1)
Ceftriaxone	138(91.2)	15(9.8)

*n*: individual number.

Figure 1 shows the trends of antimicrobial susceptibility patterns of *S. pneumoniae* isolates. They tend to be increasingly resistant to the commonly prescribed drugs as well as the recommended drugs of choice like ciprofloxacin and ceftriaxone which were found to be decreased susceptible in each year of the study area.



**Figure 1.** Trends of antimicrobial susceptibility patterns of *S. pneumoniae* isolates in each year at Gondar University Teaching Hospital, Northwest Ethiopia from September 2007 to January 2012.

AMP: Ampicillin; SXT: Sulfamethoxazole (Co-trimoxazole); TTC: Tetracycline; CAF: Chloramphenicol; ERY: Erythromycin; PEN: Penicillin G; GN: Gentamicin; CIP: Ciprofloxacin; CEF: Ceftriaxone.

#### 4. Discussion

The successful management of patients suffering from bacterial illness relies on the identification of the types of organisms that causes the diseases and the selection of an effective antibiotic against organisms in question[2]. Thus, findings in this study could provide information on the selection of antimicrobial agents for the treatment of *S. pneumoniae* in northwest Ethiopia.

All penicillin resistant isolates in this study were also resistant to one or more of the commonly used antimicrobials which is consistent to the findings of researches conducted in Malaysia, Ethiopia, and USA[14,16]. The rate of penicillin resistant *S. pneumoniae* found in this study (31.3%) is in line with the study conducted in USA (29.3%)[16]. However, the rate of penicillin resistant *S. pneumoniae* in our study (31.3%) is greater than the rate of penicillin resistant *S. pneumoniae* at Kota Bharu Malaysia (3%), USA (21.2%), Addis Ababa Ethiopia (17%), and Spain 25.9%[16,17] but lower than studies conducted in Hawassa, Ethiopia (64.2%), Kuwait (63%), Nigeria (83%) and 48.6% Israel[14,18,19]. The reasons might be due to the difference in study design, study period, methodology used and types as well as size of samples included in the study.

A study conducted in Italy forwarded an increase of pneumococcal resistance to erythromycin from 6% to 31.7%, which is in agreement to the increase of resistance to erythromycin from the previous study showed 11.5% to 20.3% in the present study area, respectively[18]. However, the present study is inconsistent with studies conducted

in USA, which showed (29.3%) pneumococcal resistance to erythromycin[15], Kuwait (42%)[18], Nigeria (56.6%)[20], Bangladesh (74%)[21] and Sweden (26%)[22]. The difference might due to study design and methodology difference, and majority of the studies used dilution techniques.

Different studies illustrated that growing of the third generation antimicrobial resistance, and this reference reduced the use of antimicrobial drugs that show insufficient sensitivity against *S. pneumoniae* to prevent resistance and associated treatment failure. The present findings of this study showed that 11.1% of the isolates were resistant to ciprofloxacin which is inconsistency with studies conducted in Nigeria (20%)[20] and the previous study done in Gondar, Ethiopia (16%)[23]. However, our result stands in opposition to another study conducted in Gondar, Ethiopia which depicted no (0%) resistance to ciprofloxacin[24]. This might be due to an ever increasing empirical treatment of meningitis and other infections by this antimicrobial agent.

A study conducted in Israel showed that there was an increasing rate of ceftriaxone resistance which rose from 0% to 10% of intermediate resistance in 1998–1999[25]. Results of our study revealed that 9.8% of resistance to ceftriaxone is in line with study conducted in Dhaka, Bangladesh (8%)[21] and Israel (8.6%)[19]. However, inconsistency with the study done in Kuwait showed 15%[18]. This might due to the difference in the methodology and study design used in the study period.

The emergence of multiple drug-resistances has complicated the empirical treatment of pneumococcal infections. Several surveillance programs in various

countries indicated that the proportion of drug-resistant *S. pneumoniae* isolates continues to increase worldwide[26]. In the present study, 43.2% of the isolates showed resistance to two or more antibiotic (Multidrug resistance was defined as resistance to  $\geq 2$  antimicrobial agents) which is higher than that in the study conducted in Gondar, Ethiopia (24%)[24], USA (30%)[16], Nigeria 12.7%[20], Spain 14.8%[17] and Kenya 7.6%[27]. However, it is lower than the study conducted in Hawassa Ethiopia showed 64.2%[14]. There is an exceedingly high rate of resistance of microorganisms to different antibiotics commonly prescribed[28]. This reasoning is also based on the fact that empirical treatments, overuses and indiscriminate use of antibiotics in developing countries and exhaustively uses in developed countries.

This study showed that substantial amount of commonly used drugs have demonstrated a decrease in activity on pneumococcal isolates as shown by an increase in the proportion of intermediate susceptibility and resistance to one or more of these drugs. High proportions of the isolates tend to be increasingly resistant to the commonly prescribed drugs in the area. This condition seeks greater emphasis more than ever before and appropriate measures enacted immediately. We recommended that performing of antimicrobial susceptibility tests before they are prescribed and administered empirically. Moreover, the recommended drug of choice like ciprofloxacin and ceftriaxone are found to have decreased susceptibility in *S. pneumoniae* isolates, and further study with a better design and survey of antimicrobial susceptibility at large scale should be done to draw advanced information.

Isolation of *S. pneumoniae* was merely by conventional culture methods (colony characteristics) and biochemical tests. However, inconsistency availability of the biochemical test sometimes may not differentiate the species and thus the findings are reported as *Streptococcus* species. This may have an impact on the findings of our study. In addition, the number and types of antibiotics used for susceptibility testing vary from time to time, and susceptibility test was done with disc diffusion methods. These may have a direct effect on our findings.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

This work was supported by Research and Community Services of University of Gondar with Grant No. RPO/55/43/2008. The authors are indebted to the University of Gondar Teaching Hospital Laboratory, Bacteriology section staff for their support and facilities during this the study.

## Comments

### Background

Antimicrobial resistance among *S. pneumoniae* continues to be a serious healthcare concern. Since 1980s, a dramatic increase in antibiotic resistance among *S. pneumoniae* has been observed in many parts of the world, especially for the frequently utilized beta-lactams, macrolides and fluoroquinolones. Consequently, there is a need to survey national and local changes in antimicrobial susceptibility patterns in order to monitor the increase in resistance, provide better therapy for patients, and maintain the effectiveness of available drugs.

### Research frontiers

This study demonstrated an increase in the proportion of intermediate susceptibility and resistance to one or more of commonly used drugs. High proportions of the isolates tend to be increasingly resistant to the commonly prescribed drugs in the study area.

### Related reports

Trends in antibacterial resistance among *S. pneumoniae* isolates which showed only penicillin and erythromycin resistance among pneumococcal isolates has remained high (Stephen *et al.*, 2008). In this study, the recommended drugs of choice like ciprofloxacin and ceftriaxone were found to have decreased susceptibility in the study area. This may be due to the fact that empirical treatments, overuses and indiscriminate use of antibiotics in developing countries and exhaustively uses in developed countries.

### Innovations and breakthroughs

In this study, 43.2% of the isolates showed resistance to two or more antibiotic (Multidrug resistance was defined as resistance to  $\geq 2$  antimicrobial agents) which is higher than studies conducted in Gondar, Ethiopia (24%) as well as many other underdeveloped countries.

### Applications

It may be significant to know the distribution of *S. pneumoniae* and to produce a systematic data on drug susceptibility profile of these isolates in a regular base. As a result, an optimized empirical treatment policy would be designed. Therefore, this study would provide up-to-date data information on the magnitude and pattern of antimicrobial resistance of *S. pneumoniae* to the commonly prescribed antibiotics in the settings.

### Peer review

The authors have made an attempt to assess the magnitude and antimicrobial susceptibility patterns of *S. pneumoniae* isolates from various clinical specimens. The manuscript is well written and provides significant contribution and importance in the concerned area.



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