

Antimicrobial Susceptibility Pattern of Invasive Pneumococcal Isolates in North West Nigeria

Garba Iliyasu, Abdulrazaq G Habib, Aminu B Mohammad¹

Department of Medicine, College of Health Sciences, Bayero University Kano, ¹Department of Microbiology, Aminu Kano Teaching Hospital, Kano State, Nigeria

ABSTRACT

Background: An alarming increase in infections due to penicillin non-susceptible pneumococci (PNSP) has been documented in nearly all countries. Increasingly, PNSP are also resistant to other antibiotics, and a growing number of clinical failures following the use of these agents have been reported. **Aims:** To determine the resistance pattern of pneumococcal isolates from patients with invasive pneumococcal infection in North West Nigeria. **Materials and Methods:** In a cross-sectional study clinical specimens were obtained from patients with community acquired pneumonia (CAP), meningitis and bacteraemia over a 2 year period. Pneumococcus strains were identified. Isolates were tested against a panel of antibiotics using E-test strips, and interpreted according to the CLSI criteria. 0.06 µg/ml was used as break point for penicillin. Analysis was carried out using descriptive statistics; relationships determined using chi-squared or Fisher's exact tests, with $P < 0.05$ regarded as significant. **Results:** Total number of isolates was 132. Twenty-two (16.7%) of the isolates were fully sensitive to penicillin while 73 (55.3%) and 37 (28.0%) were intermediately and fully resistant, respectively. One hundred and twenty-seven (96.2%) of the isolates were fully resistant to trimethoprim–sulphamethoxazole. Eleven (8.5%) were fully resistant to amoxicillin and 104 (78.8%) and 17 (12.9%) were intermediately resistant and fully susceptible. One hundred and six (80.3%) of the isolates were fully susceptible to chloramphenicol. Resistance to penicillin was shown to infer resistance to other antibiotics. **Conclusions:** Pneumococcal resistance is common in North West Nigeria. Ceftriaxone retains excellent activity against most of the invasive isolate, while trimethoprim-sulphamethoxazole is almost uniformly resistant.

Key words: Antimicrobial, invasive, pneumococcus, resistance

INTRODUCTION

Streptococcus pneumoniae otherwise call 'pneumococcus' has remained an extremely important human bacterial pathogen since its initial recognition in the late 1800s and remained an important public health concern throughout the world. Globally it is the most common cause of community-acquired pneumonia (CAP), sporadic bacterial meningitis and bacteraemia.^[1,2] While pneumococcal disease is a significant cause of morbidity and mortality worldwide, some groups bear a disproportionate share of the burden, such as the very young, the elderly, the economically disadvantaged, Africans,^[3,4] and those with HIV/AIDS.^[5,6]

From the beginning of the antibiotic era to the mid-1970s, pneumococcus remained uniformly susceptible to all classes of antibiotics that had been active against the organism, with the possible exception of tetracycline. However, in 1977 and 1978 an outbreaks of penicillin-resistance pneumococci (PRP) were reported in Durban and Johannesburg, South Africa.^[7,8] Although they were originally called PRP, these organisms appeared to have acquired genetic material that encoded resistance to both penicillin and other commonly used antibiotics. The major mechanism of resistance involves the introduction of mutations in genes encoding penicillin-binding proteins and selective pressure is thought to play an important role. High-level penicillin resistance, macrolide resistance and multidrug-resistance often complicate the management of pneumococcal infection and make choosing empiric antimicrobial therapy for suspected cases of meningitis and pneumonia increasingly difficult. An alarming increase in infections due to penicillin non-susceptible

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Address for correspondence:

Dr. Garba Iliyasu, E-mail: ilyasug@yahoo.com

pneumococci (PNSP) has been documented in nearly all countries in the world.^[9,10] Benbachir *et al.* reported 30.4% of pneumococci as non-susceptible to penicillin in four African cities (Abidjan, Casablanca, Dakar, Tunisia).^[11] In Kano, North-West Nigeria, 93%, 92%, 84%, 53% and 21% of pneumococci were non-susceptible to penicillin, co-trimoxazole, tetracycline and ampicillin, respectively.^[12] Penicillin non-susceptible rates of 18%, 29.72%, 11.76% and 67% were also reported by different workers from different regions in Nigeria.^[13-16] The rapid emergence of antibiotic resistance is adding to the burden of pneumococcal disease by significantly contributing to the numbers of treatment failures and deaths from this disease.

The previous studies in North-West Nigeria used disc diffusion method which is inadequate in distinguishing intermediate resistance from absolute resistance among pneumococcal isolates.^[17,18] However, an E-test has been shown to be a good alternative for testing pneumococcal MIC when compared with the standard agar dilution method.^[19] Hence, the main objective of the present study was to determine the resistance pattern of pneumococcal isolates from patients with invasive pneumococcal disease (IPD) in North West Nigeria using E-test strip.

MATERIALS AND METHODS

The study was a cross-sectional study among patients with community acquired pneumococcal pneumonia, meningitis or bacteraemia. The study was conducted in a tertiary referral centre in North West Nigeria over the period June 2009 to January 2011. The hospital is among the major referral centers for North-West states of Nigeria and neighboring countries like Niger republic. It has 550 beds and offers specialist inpatient and outpatient care, across various specialties.

All adult patients who were admitted with features compatible with case definition of community acquired pneumonia, meningitis and bacteraemia were recruited consecutively into the study. A sputum specimen was collected in a clean, sterile container from patients with clinical evidence of pneumonia. Blood samples were collected from all the patients, and inoculated directly into each of brain–heart infusion and thioglycolate culture media, with the use of standard aseptic procedures for aerobic and anaerobic cultures, respectively. A lumbar puncture was conducted on all the patients presenting with clinical features suggestive of meningitis if there were no contraindication, the CSF samples were collected in a clean, sterile container and was sent to the laboratories for microbiological analysis and for glucose and protein

measurement. A blood sample was taken for random plasma glucose measurement just before the lumbar puncture was performed, for comparison with the CSF glucose level. All samples were taken before administering antibiotic whenever feasible and transported to the laboratory immediately.

The laboratory operates 24 hour per day and able to process CSF specimen within 30 min of receipt. All the sputum specimen and CSF sample were inoculated onto 5% sheep blood agar and incubated at 37°C. Inoculated plates were incubated in a candle jar so as to create a reduced oxygen tension (5-10% additional CO₂ tension). Inoculated blood culture bottles were incubated in the laboratory at 37°C and observed for bacterial growth within 24 to 72 hours and at day 7 if there was no bacterial growth. Subcultures of inoculated media were done twice, on days 2 and 3 after incubation, and were inoculated onto blood agar plates and incubated as far CSF and sputum. Plates were examined after incubation for bacterial pathogens, by the use of standard procedures. Samples of all typical pneumococcal colonies obtained from the plates were subjected to pneumococcal identification methods of α -hemolysis, colony morphology and ethylhydrocupreine hydrochloride (optochin).

Microbial susceptibility tests was carried out on all confirmed pneumococcal isolate to Penicillin G, Amoxycillin, Amoxycillin–Clvulanic acid, Cefuroxime, Ceftriaxone, Tetracycline, Trimethoprim/sulfamethoxazole (TPM/SMX), Azithromycin, ofloxacin and Chloramphenicol using Etest strips (Manufactured by AB BIODISK, Sweden). Minimum inhibitory concentrations (MICs) were measured and strains were divided into resistant, intermediate or sensitive according to the CLSI guidelines.^[20]

Pneumococcal pneumonia was defined based on clinical findings plus a chest radiograph consistent with pneumonia, in addition to Gram positive diplococci on microscopy and positive culture of *pneumococcus* from an ideal sputum specimen defined as the presence of more than 25 white cells and less than 10 squamous epithelial cells per low power field. Pneumococcal meningitis was defined as isolation of *pneumococcus* with or without appearance of gram positive diplococci on microscopy from CSF or a CSF pleocytosis (>5 white blood cells/mm³) plus a blood culture positive for *pneumococcus* in a patient with clinical evidence of meningitis. Pneumococcal bacteremia was defined as isolation of *pneumococcus* from blood only.

Ethical clearance was obtained from the ethics committee of AKTH.

Analysis was carried out using descriptive statistics with differences and relationships determined using Student t-test, chi-squared and Fisher's exact tests as appropriate, with $P < 0.05$ regarded as significant.

RESULTS

The ages of the patients ranged from 18 to 82 years, with a mean age of 42.7 years \pm 18.74. The peak age groups affected were 55-64 years and ≥ 65 years. There were 72 (54.5%) males and 60 (45.5%) females with a male to female ratio of 1:2.

A total of 132 pneumococcal isolate were recovered from different clinical specimens. One hundred and seventeen (88.6%) were recovered from sputum, 7 (5.3%) from CSF and 8 (6.1%) from blood.

Twenty-two (16.7%) of the pneumococcal isolates were fully sensitive to penicillin while 73 (55.3%) were intermediately resistant and 37 (28%) were fully resistant. Only 5 (3.8%) of the isolates were sensitive to cotrimoxazole. The susceptibility profile of amoxicillin and amoxicillin/clavulanic acid was the same with 104 (78.8%) of the isolate been sensitive while 17 (12.9%) and 11 (8.5%) were intermediately resistant and fully resistant, respectively [Table 1]. Of the 132 isolates, 19 (14.4%) were resistant to only one class of antibiotic, 42 (31.8%) were resistant to two classes antibiotics while 71 (53.8%) were resistant to three or more antibiotics; none of the isolate was susceptible to the entire antibiotic tested. Penicillin-resistant pneumococcus is also resistant to more other antibiotics compared to penicillin susceptible pneumococcus [Table 2].

DISCUSSION

The pattern of prescription of drugs by non-qualified personnel, under dosing, over prescription and outright fake drug racketeering has become profound in Nigeria.^[21-23] These factors lead to development of resistance by bacteria to drugs that otherwise would have been helpful in treating infection.^[24] We demonstrated pneumococcal resistance to commonly used antibiotics in a tertiary referral center in north-west Nigeria [Table 1].

In this study 28% of the isolate were fully resistance to penicillin and 55.3% were of intermediate resistance. Similarly, a previous study using disc diffusion method reported 95% of pneumococcal isolate to be non-susceptible to penicillin in Kano, Nigeria.^[12] Akpede *et al.*^[16] in Maiduguri north-east Nigeria reported penicillin non-susceptible rate of 67% among pediatric pneumococcal

Table 1: Susceptibility pattern of the pneumococcal isolates

Antibiotics	Sensitive (%)	intermediate (%)	resistant (%)
Penicillin	22 (16.7)	73 (55.3)	37 (28)
Amoxycillin	104 (78.8)	17 (12.9)	11 (8.5)
Amoxy/clav	104 (78.8)	17 (12.9)	11 (8.5)
Cefuroxime	67 (50.8)	30 (22.7)	35 (26.5)
Ceftriaxone	127 (96.2)	—	5 (3.8)
Azithromycin	84 (63.6)	14 (10.6)	34 (25.8)
Tetracycline	17 (12.9)	18 (13.6)	97 (73.5)
Ofloxacin	81 (61.4)	34 (28.8)	17 (12.9)
TPM/SMX	5 (3.8)	—	127 (96.2)
Chloramphenicol	106 (80.3)	1 (0.8)	25 (18.9)

Amoxy/Clav: Amoxicillin/clavulinate, TPM/SMX: Trimethoprin-Sulphamethoxazole

Table 2: Resistance pattern of PRP and PSP against other antibiotics

Antibiotics	PRP Isolates n = 37 (%)	PSP Isolates n = 22 (%)	X ² value	P Value
Amoxycillin/ clavulanic acid	10 (27.0)	0 (0.0)	7.16	0.01*
Cefuroxime	29 (78.4)	2 (9.0)	26.56	<0.001*
Azithromycin	13 (35.1)	1 (4.5)	7.13	0.01*
Ofloxacin	5 (13.5)	3 (13.6)	0.0002	1.00*
Erythromycin	19 (51.4)	3 (13.6)	8.39	0.01*
Co-trimoxazole	37 (100)	19 (86.4)	5.32	0.047
Tetracycline	26 (54.1)	18 (81.8)	0.97	0.37
Chloramphenicol	17 (45.9)	3 (13.6)	6.43	0.01*
Ceftriaxone	1 (2.7)	0 (0.0)	0.005	1.00*

*Fisher's exact test, PRP: Penicillin-resistant pneumococci, PSP: Penicillin-susceptible pneumococci

isolates, while in north central Nigeria Akanbi *et al.*, in Ilorin reported resistance rate of 83%,^[25] which is similar to our report. Furthermore, a recent multicenter study in two major cities in south west Nigeria reported 100% of pneumococcal isolates to be resistant to penicillin,^[26] however, a decade earlier a resistance rate of 36%^[27] was reported from this region thus highlighting the rapid spread of PRP in Nigeria. A lower resistance rate of 29.7% and 11.76% were reported among non-invasive nasopharyngeal isolate in North Central and South West Nigeria, respectively.^[14,15] The high prevalence of PRP in Nigeria is not surprising as penicillin are the most widely used antibiotics. The greater the quantity and the longer the duration an antibiotic have been in use, the more likely resistant strains emerge to that particular antibiotic.^[28]

The high prevalence of HIV infection and an attending prophylactic usage of TMP-SMX in AIDS patients has been linked to the wide spread pneumococcal resistance to TMP-SMX.^[29] This antibiotic had been recommended by WHO for the treatment of pneumococcal disease in HIV/AIDS patients.^[30] We found high prevalence of TMP-SMX (96.2%) resistance which is comparable to

report from different part of Nigeria,^[12,31,32] even though some studies in Nigeria reported a lower figure.^[26,27] Amoxicillin and Amoxicillin/clavulanic acid showed relatively good activity in this study with 78.8% of the isolates being sensitive while an excellent activity was seen with ceftriaxone, with only 5% being fully resistance. Other Nigerian studies also reported a good susceptibility rate to these antibiotics.^[12,22,27,32] However, contrary to our findings Bamidele *et al.*^[26] reported a resistance rate of 55.6% among invasive pneumococcal isolates to amoxicillin–clavulanate, while Akanbi *et al.*^[25] in a hospital-based study reported a resistance rate of 28.0% to ceftriaxone. This excellent activity seen with ceftriaxone might be related to the cost of the drug which is prohibiting in Nigeria, therefore not usually abuse. The macrolides have long been important in treating CAP, because of their excellent activity against pneumococci. This activity has been eroded in the past few years by the proliferation of macrolide-resistant strains. The prevalence of these resistance strains continues to increase in many parts of the world. In this study the resistance rate to azithromycin was 25.8%. A higher resistance rate of 56.6% and 66.7% to erythromycin were reported by other workers in Nigeria.^[25,26] Contrary to our findings, Onipede *et al.*^[33] reported 100% of invasive pneumococcal isolates from children to be sensitive to erythromycin which is also similar to findings by Adeleye *et al.*,^[32] among sputum isolates from HIV-infected pediatrics and adult patients. Chloramphenicol is an old antibiotic that has been so much abused in the past, as a first-line treatment for typhoid fever in most developing countries, however, with the appearance of cheaper generic forms of quinolones, and appearance of chloramphenicol-resistant *Salmonella typhi*, prescribers have moved away from chloramphenicol and this relief of pressure on the drug, may be the reason for the good performance of chloramphenicol in this study with 80.3% being fully susceptible, with a reciprocal increase in ofloxacin non-susceptibility (41.7%). This corroborate with findings by other workers in Nigeria.^[25,26,32]

It has been reported that pneumococci resistant to penicillin are more likely to become resistant to other antibiotic than penicillin-susceptible strains.^[34] This is shown in this study where the resistance percentages among the cefuroxime, erythromycin, chloramphenicol, amoxicillin–clavulanic acid and azithromycin were consistently higher among penicillin-resistant strains than among penicillin-susceptible isolates [Table 2]. Kandakai *et al.* also reported similar findings.^[14]

The heavy burden of community-acquired infectious diseases in most developing countries dictates a heavy requirement for antibiotics, often empirically.

Chloramphenicol and TPM/SMX are commonly use as first-line drugs in the integrated management of childhood illness (IMCI) scheme.^[22] In addition to TPM/SMX, penicillin and erythromycin are also widely use in sickle cell anemia and HIV/AIDS patient to prevent opportunistic infections.^[35] These coupled with wide spread antibiotic self-medication,^[23,36] may be promoting rapid emergence of antibiotic resistance in Nigeria.

In the first-line treatment of severe invasive pneumococcal infection in our environment we recommend the use of ceftriaxone; however, in a resource-constrained settings chloramphenicol, amoxicillin or amoxicillin–clavulanic acid may be an alternative. Cefotaxime was not studied in this study however it is expected to have similar spectrum to ceftriaxone, hence may be considered were indicated. With high prevalence of penicillin resistance in this study the use of penicillin for empiric treatment of suspected pneumococcal infection is no longer recommended. The findings of our study even though limited by small sample size also underscored the need for a comprehensive action plan in Nigeria to convert rapid spread of antimicrobial resistance among common bacteria pathogens such as pneumococcus and its associated morbidity and mortality. These should include promotion of rational use of antimicrobials, strengthening of antimicrobial resistance surveillance, and antimicrobial legislation among others.

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