AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JAN 2004 ISSN 1595-689X VOL.5 NO.1
AJCEM/2003033/2409

PENICILLIN-RESISTANT STREPTOCOCCUS PNEUMONIAE – A REVIEW

Taiwo, S. S., Onile, B. A.

**Department of Medical Microbiology,
University of Ilorin Teaching Hospital, P. M. B. 1459, Ilorin, Nigeria**

Correspondence to: Dr S. S. Taiwo (E-mail: samtaiwo2003@yahoo.com)

Since the first report in 1967, the incidence of Penicillin Resistant *Streptococcus pneumoniae* (Pneumococcus) has risen steadily worldwide, and now complicates diagnostic and treatment strategies for infections due to this organism. More worrisome is the fact that in areas where Penicillin Resistant *Streptococcus pneumoniae* (PRSP) has become established, resistance to other antimicrobial agents such as cephalosporins, sulphonamides and macrolides is also common. This development has a grave implication for therapy of life threatening pneumococcal infections like meningitis and septicaemia, with the extended spectrum cephalosporins, such as ceftriaxone and cefotaxime, and the newer macrolides, azithromycin and clarithromycin. The mechanism of resistance to β-lactam antibiotics is decreased binding of drug to the bacteria cell wall brought about by genetic transformation in bacterial chromosome. Recently, using molecular techniques that can index overall relatedness of the drug resistant pneumococcal isolates, it has been possible to establish clonal dissemination of drug resistant pneumococci across continents, with acquisition of additional drug resistance determinants as a result of "local" antibiotic selective pressures. This is a review of literature on the epidemiology, mechanism of resistance, laboratory identification, treatment, prevention and control of Penicillin Resistant Pneumococci (PRP), with emphasis on the problems of identification and reporting in developing countries.

Key words: penicillin, *Streptococcus pneumoniae*, resistant, extended spectrum cephalosporins.

INTRODUCTION

Streptococcus pneumoniae infections are among the leading causes worldwide of illness and death in young children, persons with underlying illness, and the elderly. In the United States of America alone, *Streptococcus pneumoniae* is a leading cause of morbidity and mortality, resulting each year in an estimated 3,000 cases of meningitis, 50,000 cases of bacteraemia, 500,000 cases of pneumonia and 7,000,000 cases of otitis media (1, 2). In developing countries, 50% of the estimated 4 million child deaths annually from

pneumonia are caused by *Streptococcus pneumoniae* (3).

In the past, *Streptococcus pneumoniae* was almost uniformly susceptible to penicillin, allowing most physicians to treat persons who had severe infections with penicillin alone and without testing for resistance. Since the 1960s however, resistance to penicillin and most other antimicrobial agents, has spread rapidly. Penicillin Resistant *Streptococcus pneumoniae* (PRSP) was first reported in 1967 in Australia (4), in New Guinea in 1969 (5) and in South Africa in 1977 (6), and since then in many countries throughout Africa, America, Asia and Europe (7-26).

In Africa, with the exception of South Africa, literature on the occurrence of PRSP appears sparse. The few reports have documented low prevalence rate, which is probably due to gross underreporting. Poor or absent antibiotic resistance surveillance and infection control progammes, and poor laboratory backup in many health institutions are some of the factors responsible for underreporting of the occurrence of PRSP and other resistant organisms in these countries. This article reviewed the epidemiology, diagnostic and therapeutic difficulties, and preventive measures for PRSP and emphasizes the need to increase surveillance for these organisms in developing countries.

HISTORICAL/EPIDEMIOLOGICAL PERSPECTIVES

The German pathologist, Klebs in 1875 described *Streptococcus pneumoniae* in the fluid from the lungs of a man dying with pneumonia (27). In 1881, the organism was concurrently identified in the old and new worlds, by Pasteur in France, who named it *Microbe Septicemique du salive*, and Sternberg in the United States, who called it *Micrococcus pasteuri* (27,28). By late 1880s, the term pneumococcus was generally used because this bacterium has come to be recognized as the most common cause of lobar pneumonia (28). It was also recovered from several body sites such as cerebrospinal fluids, synovial fluids, kidney, middle ear, blood and the pericardium (27,28).

In 1890s, Felix and klumperer showed that immunization

with killed pneumococci protected animals against subsequent pneumococcal challenge, and further, that protection could be transferred by infusing serum from immunized mice into naive recipients (29). Felton prepared the first purified pneumococcal capsular polysaccharides for immunization of human subjects (30), and a preparation of type 1 polysaccharide was used to abort an epidemic of pneumonia at a State Hospital in Worcester, Massachusetts in 1938 (31). MacLeod and coworkers further confirmed this during the World War II when they found that vaccinating military recruits with capsular material from several serotypes of *Streptococcus pneumoniae* greatly reduced the incidence of pneumonia due to serotypes present in the vaccine, but not other pneumococcal serotypes (32). Serotypes of pneumococci were earlier recognized, based on the observation that injection of killed organism into a rabbit stimulated the production of antibody that agglutinated and caused capsular swelling of the immunizing strains, as well as of some, but not all, other pneumococcal isolates (29). Capsule swelling (Quellung) reaction became the basis of the American and Danish serotyping schemes of *Streptococcus pneumoniae.*

The name Diplococcus was adopted in 1926 based on the presence of paired cocci on Gram stained sputum. In 1974 it was renamed *Streptococcus pneumoniae* based on the presence of chains when grown in liquid medium (27). *Streptococcus pneumoniae* was the subject of pioneering genetic research work by Griffith (33), when in 1928, he demonstrated genetic transformation in *Streptococcus pneumoniae.* In the 1930s, it was recognized that a large proportion of healthy population carried pneumococci in the nasopharynx and that this was often a source of disease in contacts of these asymptomatic carriers (28,34).

In the pre antibiotic era, mortality from untreated pneumococcal disease was 77% (35). It was 50% in patients treated with specific antipneumococcal serum (35). Following the introduction of sulphonamides in 1930 and penicillin in 1945, mortality decreased to about 25% (36) but remained unchanged at this rate for about three decades of antibiotic therapy (37,38,39).

During this period (1940s to 1970s) however, occasional strains of pneumococcus exhibiting increased resistance to penicillin (4,40), tetracycline (41,42), erythromycin and lincomycin (43,44) had emerged.

The first documented evidence of resistance to pneumococcus was in 1912 by Morgenroth and Kaufman (45) when optochin (ethylhydrocuprein hydrochloride) resistant pneumococci were obtained from experimentally infected mice treated with optochin. Optochin resistant pneumococci were then reported among clinical isolates obtained from patients treated with optochin in 1915 (46). Sulphonamide resistant pneumococci were reported in patients with meningitis and lobar pneumonia between 1939 and 1943 (47). Penicillin resistance was first described in 1945 among mutant strains of pneumococci *in vitro* (40) shortly after the introduction of penicillin into the market. Clinical isolates of *Streptococcus pneumoniae* with reduced susceptibility to penicillin were first reported in Boston in 1965 (48). Penicillin minimum inhibitory concentration (MIC) of 0.1 and 0.2 µg/mL were reported in two of the two hundred isolates tested. Despite these findings, the investigators failed to recognize the clinical significance of their discovery. The first pneumococcal strain with reduced susceptibility to penicillin (MIC 0.6 µg/mL), for which clinical relevance was recognized, was reported in 1967 (4) after being isolated from a 25-year old Australian woman. During the next decade, several alarming reports were published documenting worldwide spread of pneumococci with reduced susceptibility to penicillin (MIC 0.1 – 1 µg/mL).(5-9). In 1977 (10), pneumococci exhibiting high level penicillin resistance (MIC ≥ 2.0 µg/mL) were isolated from young African children admitted to King Edward VIII Hospital, Durban, South Africa, with meningitis, septicaemia, otitis media, pneumonia and empyema. By the early 1980s, worldwide distribution of multidrug resistant pneumococci have been described with reports from New Guinea, Israel, Poland, South Africa and the United States of America (11-15).

The incidence and pattern of penicillin resistance among pneumococci remained fairly stable in the early 1980s (14), but due to the various degree of resistance en-

countered and the various nomenclatures used, the Centre for Disease Control and Prevention (CDC), in 1995 suggested a standardized classification of resistance level (49). CDC defines susceptibility of *Streptococcus pneumoniae* to Penicillin as MIC ≤ 0.06 µg/mL. All isolates for which the MIC is ≥ 0.1 µg/mL are regarded as non-susceptible. Isolates that are non-susceptible are characterized further as Penicillin intermediate (*Peni*; MIC 0.1-1 µg/mL) or Penicillin resistant (*Penr*; MIC ≥ 2.0 µg/mL). Isolates for which MIC is ≥ 2.0 µg/mL were previously referred to as displaying high-level penicillin resistance (50). This terminology is no longer advocated by CDC (49).

Between 1979 and 1987, non-susceptible pneumococci accounted for approximately 5% of the strains recovered in the United States. During the same period, Penicillin resistant strains (*Penr*) were rare, approximately 0.02% (1 of 4585) of pneumococci sterile-site isolates submitted to the CDC Sentinel Hospital Surveillance system (51). By the early 1990s, however, a dramatic increase in the frequency of isolation of non-susceptible pneumococci was reported (52-57), with a corresponding increase in Penicillin resistant (*Penr*) strains. For example in 1991 – 1992, 2.6% of all isolates were Penicillin resistant as against 7.3% in 1992-93 (52, 53) and 9.5% in 1994-95 (57). Similarly, several other countries reported increasing incidence of Penicillin non-susceptible strains with corresponding increase in Penicillin resistant (*Penr*) strains during this period (16-26). The common serotypes of pneumococci resistant to penicillin (MIC ≥ 2.0 µg/mL) and other β-lactam agents encountered include serotypes 6A, 6B, 19A, 19F, 14 and 23 (13). Others include serotypes 1, 3, 5, 15, 31 and 35 (15). In the United States, outbreaks in daycare centres were caused mainly by serotypes 6B, 14, 19F and 23 (58, 59).

In Africa, only few surveys have been reported except in South Africa, where resistant rates are close to 20% (60). Surveys carried out in Nairobi, Kenya in 1981 (61) and during 1991 to 1992 (62) gave 26% prevalence rates. In Tunisia, a rate of 10% was reported (63). Reports from Zambia (64), Senegal and Ivory Coast (63) have posted

rates below 5% and less than 2% in Morocco and Egypt (63). A survey in Nigeria in 1978 reported a 20% prevalence rate for Penicillin Resistant Pneumococci (65). The relatively few surveys carried out by African countries do not give a true picture of the occurrence of PRSP in this continent. More surveys will be necessary to know the true prevalence in these countries.

Penicillin Resistant Pneumococci have been recovered more frequently from children five years or younger than from other age group (1, 2, 9-13,64,67,68,73,74). Although young children are still at risk for resistant infection, an increased frequency of drug resistant pneumococci has been encountered in adults (60). Risk factors for an infection secondary to a resistant pneumococcal strain include hospitalization, prior exposure to antimicrobial agents, underlying illness and tobacco use (6-13,15,19,23,39,67,68,69,71).

In areas where PRSP has been established, resistance to other antimicrobials, such as cephalosporins (67-71), sulphonamides (15, 60, 73) and macrolides (66, 74) is also common. The identity of drug-resistant isolates within a country or in different countries has been investigated using techniques such as polymerase chain reaction (BOX PCR), pulse field gel electrophoresis (PFGE), multilocus enzyme electrophoresis (MLEE), penicillin binding protein (PBP) profiling and multilocus sequence typing (MLST) to index their overall relatedness (75, 76). This has illustrated the extreme diversity of drug resistant pneumococci particularly in countries such as Spain and Africa, where resistant isolates have emerged rather than being imported. However, superimposed on this diversity is the emergence and clonal spread of resistant isolates that are presumably fitter than other isolates. Thus in Spain although there is a great divergence in the relatedness of resistant isolates, more than 60% of all Penicillin resistant isolates belong to four major clones; Spain^{23F}-1, Spain^{6B}-2, Spain^{9V}-3 and Spain^{14}-5 (77). Two of these clones have been extensively studied. The first major clone, Spain^{23F}-1 (resistant to penicillin, tetracycline, chloramphenicol and sometimes erythromycin) probably arose from Spain in the early 1980s and since then has spread across more than six other

countries in three continents (78). The spread of this clone has been accompanied by the emergence of variants that have acquired additional drug resistance determinants as a result of "local" antibiotic selective pressures. The second major Spanish clone, Spain^{6B}-2 is also resistant to penicillin, tetracycline and chloramphenicol and has become prevalent in Iceland and the United Kingdom (79), other European countries (80,81) and in Asia (82). Extensive human population mobility is the major factor in the global dissemination of these resistant clones.

MECHANISM OF RESISTANCE IN PRSP

Pneumococcal resistance to β-lactam agents, like Penicillins and Cephalosporins, is due to changes in the target sites of the enzymes called Penicillin Binding Proteins (PBP). These high molecular weight proteins are believed to catalyze the terminal stages in peptidoglycan (murein) synthesis (83). There are six PBPs found in susceptible strains of *Streptococcus pneumoniae;* PBP 1a, 1b, 2a, 2x, 2b and 3 (84). All are high molecular weight proteins except PBP3, which is probably not too involved in β-lactam mediated cell lysis (84, 85). β-lactam compounds inhibit these enzymes by covalently binding to their active sites (84).

The altered PBPs in pneumococcus have low affinity for penicillin and related β-lactam compounds (85), a mechanism which only occurs in organisms that are naturally transformable. Altered PBPs also play a role is resistance to Penicillin in other naturally non-transformable strains like *Staphylococcus aureus* and enterococci (86), but in these cases, it is due to acquisition of new abnormal PBPs, rather than decrease in the affinity of the normal PBPs. Pneumococcal isolates with high Penicillin MIC seems to be entirely due to the expression of low affinity forms of PBP 1a, 2a, 2b, 2x and perhaps 1b (87). There is a reduction in the affinity of at least three of these five PBPs. For example, resistance to at least 8 μg/mL can be achieved by alterations in only PBP 1a, PBP 2x and PBP 2b. Since PBPs in these strains also have decreased affinities for other β-lactam antibiotics, most PRSP have increased resistance to third generation cephalosporins including ceftriaxone and cefotaxime. High- level resistance

to cephalosporins requires reduction in the affinities of only PBP 2x and PBP 1.

The reduction in the affinity of PBPs for β-lactam compounds results from the appearance of the so-called "mosaic" amino acid sequences of the proteins (85). Over the last few decades, the high selective pressures provided by antibiotics in the environment of the bacteria have selected for strains that have these new or changed PBPs, less able to bind β-lactam antibiotics. What we see today are pneumococci that have PBP encoding chromosomal genes that are hybrid molecules made with DNA from *Streptococcus mitis* and other yet to be identified streptococcal species (88). Many different "mosaic" genes have been sequenced to date. It is difficult to calculate the exact events that gave rise to these variants PBPs. To complicate matter is the fact that these "mosaic" genes have been transferred to other viridians streptococci such as *Streptococcus sangius* and *Streptococcus oralis* (87). As a result of the gene flow between these naturally transformable streptococci, it is difficult to determine the events that occurred to produce these mosaic PBPs now found in resistant pneumococci.

PRSP with MIC ≥ 2 μg/mL are also more likely to be resistant to non β-lactam agents such as chloramphenicol, trimethoprim-sulphamethoxazole, erythromycin, tetracycline and aminoglycosides (15,60,66,74,75). Resistance to chloramphenicol, tetracycline and erythromycin appears to be chromosomally mediated (89). A 7.244 kb defective transposon Tn *1207.1* containing *mef* (A) gene has been found inserted in the competence *cel* B chromosomal region of *Streptococcus pneumoniae* conferring resistance to macrolide (M phenotype) via an active efflux mechanism (90). Another 25.3 kb conjugative transposon, Tn *1545* has also been found inserted into the chromosome of resistant pneumococcus (91). This transposon carries the *erm* (B) gene that confers resistance to erythromycin and the structurally unrelated macrolide, lincosamide and streptogramin B (MLS_B phenotype), through methylation of 23S rRNA, the common target of these agents. This transposon also carries the *tet* (M) gene

that codes for tetracycline resistance via the production of a protein that binds to the ribosome and blocks protein synthesis (92). Resistance to trimethoprim-sulphamethoxazole is attributed to trimethoprim and occurs by a decrease in the affinity of trimethoprim for its target enzyme, dihydrofolate reductase (93). Chloramphenicol resistance is due to the production of an inducible chloramphenicol acetyltransferase (94).

It appears that pneumococci, in addition to having incorporated DNA from non-pneumococcal streptococci, may have also shared DNA with Gram-negative microbes in other to acquire additional drug resistance. The genes that encode resistance to erythromycin, tetracycline and aminoglycosides, *erm* (B), *tet* (M) and *aph* (A3) respectively, identified in PRSP have also been found in *Escherichia coli* and *Klebsiella spp*, *tet* (M) in resistant *Haemophilus* and *Neisseria spp*, and *aph* (A3) in *Staphylococcus aureus*, *Enterococcus faecalis* and *Helicobacter spp* (89).

Although the quinolone antimicrobials have improved spectrum of activity against Gram-positive organisms, they do not possess sufficient activity to be clinically useful against *Streptococcus pneumoniae*. One of the genes responsible for resistance to quinolones is *gyr* (A), which encodes the A-subunit of DNA gyrase, the site of action of quinolones (95). Newer quinolones may be potent against *Streptococcus pneumoniae* despite mutations in the *gyr* (A) and *par* (C) genes and may be useful in treating infections by this organism (96).

To date, β-lactamase producing pneumococci have not been reported. The mechanism of resistance is entirely chromosomal with horizontal transfer of resistant genes via transformation and conjugation.

LABORATORY IDENTIFICATION OF PRSP

Streptococcus pneumoniae is a fastidious organism requiring particular attention to proper laboratory procedures for identification and *in vitro* susceptibility testings (97). Routinely in the laboratory, pneumococci are identified by three reactions; the so-called alpha-haemolysis on blood agar with flat or concentrically ringed colonies, catalase negativity and solubility in bile salt or susceptibility to ethyl-

hydrocupreine (Optochin). Occasional strains may form rounded, rather than flat or concentrically ringed, colonies or may lack capsules and hence mis-identified as viridian streptococci. These atypical strains are likely to be encountered from sites with normal flora or among Penicillin resistant strains (98). Strains with zone diameter of inhibition ≥ 10 mm to optochin disk can be presumptively identified as pneumococci. Incubation in air with added CO_2 caused decreased zone size, which is reversed when pneumococci, but not viridian streptococci, are incubated in air (101). In recent years, a number of isolates have been found to be optochin resistant (102,103), which has led cautious microbiologists to rely more on the use of bile solubility for definitive identification.

IN-VITRO SUSCEPTIBILITY TESTING

Recent reports have emphasized the importance of accurate susceptibility testing of all clinically significant isolates of *Streptococcus pneumoniae*, the need for new agents, and periodic revaluation of existing drugs (1,97,104). Testing is complicated by the fact that there are currently no automated microbroth dilution MIC systems available for susceptibility testing of *Streptococcus pneumoniae*.

Laboratory may choose to use agar disk diffusion on Mueller-Hinton agar supplemented with 5% sheep blood, incubated in 5% CO_2 to screen for Penicillin resistance using the 1 µg oxacillin disk (49,97,98) or 5 µg methicillin disk (99). The cut off zone diameter of inhibition for oxacillin is 20 mm (49) and for methicillin, 25 mm (99). Oxacillin is preferred to methicillin, because of the enhanced resistance of oxacillin to degradation during storage (100). This method is also acceptable for testing other oral agents including trimethoprim/sulphamethoxazole, erythromycin, clindamycin and tetracycline as well as vancomycin for parenteral use (49,105). Penicillin disk is not used because it gives inaccurate results (98).

Susceptibility to Penicillin can be used to predict susceptibility to all other β-lactams. However, to distinguish between Penicillin intermediate and Penicillin resistant isolates, and to obtain susceptibility information for cephalosporins, a quantitative MIC test must be

done (1,49,97). The agar dilution method is regarded as the reference method for determining the MIC for pneumococcus (49,98). This is carried out in cation-adjusted Mueller Hinton agar supplemented with 5% whole defibrinated horse or sheep blood or 5% lysed and centrifuged horse blood for sulphonamides (98,106). The inoculum size is 10^4 CFU per spot and plates are incubated in air or added 5-10% CO_2 overnight (49).

Recently, the E-test (AB Biodisk, Solna, Sweden) has become popular. This is a method of determining MIC based on diffusion of an antimicrobial gradient from a calibrated antibiotic impregnated plastic strip applied onto the surface of an inoculum coated agar plate. The antibiotic gradient produced results in an ellipse of inhibition. The point at which the ellipse meets the strip is the MIC. This technique has become widely used in clinical laboratories for quantifying MICs for penicillin and third generation cephalosporins (1,49,107). Evaluation of the E-test has shown excellent correlation with agar dilution and broth microdilution method for Penicillin G, Cefotaxime, Ceftriaxone, Amoxicilin, Chloramphenicol, Erythromycin and Tetracycline, though, the MIC for Penicillin G tends to be slightly lower resulting in some resistant strains being categorized as intermediate (107,108).

TREATMENT OF PRSP INFECTIONS

Opinion differs on how to treat infections caused by Penicillin Resistant Pneumococcus. There are very few randomized controlled clinical trials of antimicrobial agents for the treatment of these infections. Schreiber and Jacobs (1), in a recent review, stressed the need for more controlled trials to determine optimal antimicrobials or other intervention necessary to treat infections due to PRSP. To optimize initial or empiric therapy for pneumococcal infections, clinical health-care providers must be informed of the prevalence and patterns of resistance among isolates in their community. The degree of resistance, variability in drug levels at different sites, particularly in the CSF and middle ear, natural history of the disease at different sites and in different age groups, stage of infection at which initial or appropriate therapy was instituted and presence of underlying ill-

nesses such as malnutrition, immunodeficiency, or malignancy, are some of the factors affecting treatment outcome (15,98).

The consensus among recent reviews is that Penicillin should no longer be used in the initial treatment of pneumococcal meningitis (109-110). Several authors advocate monotherapy with third generation cephalosporin, either ceftriaxone or cefotaxime (110-112), while others suggests initial therapy should include the combination of ceftriaxone or cefotaxime with vancomycin (111,112). A clinical study by Viladrich *et al* in Barcelona, Spain (114) showed cefotaxime and ceftriaxone to be reasonable first agent for meningitis. Although, Penicillin was potentially effective against sensitive strains or even intermediate resistant strains in high doses, they recommended that it should not be used as first line agent in view of the poor clinical outcome in their patients. Vancomycin has also been evaluated for the treatment of PRSP associated meningitis (115), but concern about penetration into the cerebrospinal fluids in adults prompted studies of combination regimens. Vancomycin and ceftriaxone combination was found to be synergistic even against strains with high penicillin and cephalosporin MIC (116). Ceftriaxone and rifampin was also found to be effective in adults given dexamethasone as adjunctive therapy (117). In adults treated with adjunctive dexamethasone, ceftriaxone plus rifampin is the preferred empiric combination regimen because dexamethasone reduces the penetration of vancomycin into the CSF in adults but not in children (118).

In areas with low prevalence of Penicillin Resistant Pneumococci therefore, empiric initial therapy with a third generation cephalosporin is advocated. In areas where pneumococci resistant to extended spectrum cephalosporins are prevalent, empiric therapies with vancomycin and an extended spectrum cephalosporin should be considered, until culture and susceptibility results are known. If the Penicillin MIC for the agent is < 0.1 µg/mL, then therapy can be changed to Penicillin 500,000 units/kg/day alone, which will most often be less expensive and carry less risk of promoting resistance to third generation cephalosporin and vancomycin. Alterna-

tively, the cephalosporin may be continued alone. For intermediate resistant isolate (MIC 0.1-1.0 µg/mL), third generation cephalosporins should be considered alone with vancomycin discontinued. When the MIC equals or exceeds 2.0 µg/mL or when there is little or no clinical improvement, the combination of cephalosporin and vancomycin should be continued. Vancomycin should not be used alone in the treatment of *Streptococcus pneumoniae* associated meningitis (115). Also, chloramphenicol is no longer recommended for use in the treatment of pneumococcal meningitis. Friedland and Klugman (119) demonstrated unfavourable outcome, defined as death, severe neurologic deficit or poor clinical response in 80% (20 of 25) of patients with PRSP meningitis treated with Chloramphenicol, 75-100 mg/kg/day, as initial therapy. Similarly in Dallas, 12 of 16 penicillin resistant isolates of *Streptococcus pneumoniae* from blood or CSF were associated with chloramphenicol minimum bactericidal concentration (MBC) of 8 µg/ml or more, resulting in poor clinical response (120).

In the treatment of otitis media due to PRSP, the elevated MIC for oral β-lactam agents including the new cephalosporins, the relatively low serum concentrations and poor penetration of antimicrobials into the middle ear combined to complicate therapy of otitis media due to these organisms (1, 119, 121). Amoxicillin has been advocated as the drug of choice for the initial treatment of acute otitis media, even in regions with high prevalence of PRSP (109,110,125). Studies have demonstrated relatively high clinical success rate in patients with PRSP associated otitis media ranging from 63% with Amoxicillin in a rural Kentucky study (122) to 82% with Amoxicillin Clavulanate potassium in another large multicentre open labeled trial in the United States of America (123). The clinical efficacy of second-generation cephalosporins, cefuroxime axetil and cefprozil, against pneumococci, have also been demonstrated in some studies (124-126). Barry *et al* (125) recorded 81% (43 of 53) clinical success rate in children with PRSP associated acute otitis media and 92% (152 of 166) in PSSP group.

Gehanno *et al* (126) also reported success rate of 75% for Penicillin resistant strains, 90% for Penicillin intermediate strain and 93% in Penicillin susceptible strain of pneumococcal acute otitis media in children under five years of age. Based on these studies, it is advocated that Amoxicillin 40 mg/kg/day should be the first line agent in the empiric treatment of acute otitis media in children and adults. In children with recurrent otitis media, who have not responded to Amoxicilin, Amoxicillin-Clavulanate (40 mg/kg/day Amoxicillin and 10 mg/kg/day Clavulanate) or second-generation cephalosporin, such as cefuroxime axetil (30 mg/kg/day) should be considered. For strain refractory to oral agent, injectable second or third generation cephalosporin or vancomycin may be indicated.

In treating Pneumococcal pneumonia due to resistant organism, opinion also differs over the best initial agent. This is as a result of few controlled trials designed to document the outcome in these patients. In a study by Pallares *et al* (127) and the report of the American Thoracic Society (128), underlying disease appeared to be a more significant risk factor for mortality than the susceptibility to the infecting organism. Hence some authors continue to emphasize the use of injectable Penicillin as a first line agent, claiming that treatment failure is much less likely than in meningitis caused by a strain with the same level of drug resistance (111, 129, 130). Others recommend initial use of cefuroxime, cefotaxime or ceftriaxone (109,111). Based on the available literature (109,111,113,128), it is currently advocated that initial treatment of pneumococcal pneumonia in patients requiring hospitalization should consist of cefuroxime, ceftriaxone or cefotaxime. Therapy can be altered on the basis of the clinical response and not solely on the MIC. If a patient is infected by a non-susceptible strain but is responding to treatment, no change in antimicrobial therapy is necessary. In patients with underlying disease or in community with high prevalence of PRSP, initial therapy should consist of cefotaxime or ceftriaxone and vancomycin. Therapy may be changed depending on the susceptibility of the organism and patient's clinical response.

CLINICAL SIGNIFICANCE OF PRSP INFECTION

The clinical importance of Penicillin resistance among pneumococci appears largely uncertain. Some reports (127,130) seem to suggest that patient outcomes are similar in individuals with PRSP and PSSP infection, even when the initial therapy consists of a β-lactam antibiotic. Older age and underlying disease appears to be more important factors influencing death from invasive pneumococcal disease than β-lactam susceptibility (127,128,131).

However, increase dosages of β-lactam agents is required to produce adequate bactericidal concentration (in view of the elevated MIC) particularly in the CSF and middle ears. Though patient outcome may not differ significantly from sensitive cases, financial costs may be influenced by the large doses required. Patients with hospital acquired non-susceptible pneumococcal infection were shown by Weis *et al* (132) to cost an institution approximately $16,000 more to treat than patients with Penicillin susceptible bacteria ($P < 0.05$). The difference in treatment costs was attributed to increased patient care requirement such as intensive or critical care beds and nursing services. In a nutshell, infection with resistant organism tend to increase the overall cost of therapy at both individual and institutional level and also increase the risk of toxicity from the increase use of potentially toxic drugs like vancomycin.

PREVENTION OF PNEUMOCOCCAL INFECTION

Patients who are at high risk of acquiring infections by pneumococci such as splenectomized patients, sickle cell anaemia patients, patients with immunoglobulin deficiencies or haematological malignancies should benefit from prophylactic Penicillin V or Erythromycin (98). Bacteraemia with PRSP may however occur in these groups of patient (133).

The use of multivalent polysaccharide vaccines in selected groups such as the elderly has been recommended. The 14-valent pneumococcal vaccines is no longer in use because of its lack of efficacy in children under 2 years of age and only 64% efficacy in children greater than 2 years (134). The currently available 23-valent pneumococcal vaccines contain purified

capsular polysaccharide antigens from 23 serotypes of *Streptococcus pneumoniae,* representing 85-90% of the serotypes responsible for invasive disease in children and adults in the United States (135,136). Of the seven serotypes most commonly associated with drug resistance, six are represented in the vaccine. Some degree of protection is provided against serotype 6A that is absent in the vaccine because of serologic cross-reactivity with serotype 6B that is present in the vaccine (137). Because of the emergence of drug resistant pneumococcal infection, there is need for adherence to the recommendation of the Advisory Committee on Immunization Practices (ACIP) that persons 2 years and above, with medical conditions placing then at increased risk for pneumococcal infection and all persons 65 years and above, should receive the 23-valent pneumococcal vaccines (138,139).

Children under the age of 2 years are especially susceptible to invasive pneumococcal infections and are at an increase risk for drug resistant infection. Commercially available polysaccharide vaccines are not able to elicit adequate immune response in young children under 2 years of age. This has led to the development of pneumococcal capsular polysaccharide-protein conjugate vaccine that employ the same principle used in *Haemophilus influenzae* type b vaccine; coupling the polysaccharide to a carrier protein, which increases immunogenicity (140,141).

Preliminary antibody titre result shows these vaccines, containing many serotypes, to be safe, and consistently elicit an immunologic response in infants as young as two months (140,141). In February 2000 (142), a conjugate vaccine for seven pneumococcal serotypes was licensed for use in infants and children, and is now recommended in the United States for all children less than 2 years of age, with catch-up vaccination schedules suggested for children 2 to 4 years of age (143).

OTHER CONTROL MEASURES

Surveillance for drug resistant *Streptococcus pneumoniae* should be initiated in all institutions and communities. In some states in the United States of America (56), state-wide surveillance for drug resistant *Streptococ-*

cus pneumoniae, as a notifiable condition, has been initiated. The Centre for Disease Control and Prevention (CDC), in collaboration with the Council of State Territorial Epidemiologists and Public Health Laboratory Directors, is helping to develop strategies for collecting information on PRSP in other states and for preventing morbidity and death associated with infection with these strains. Eradication of carrier states may also be an option to reduce level of resistance in community (60). Attempt at eradication with rifampicin and erythromycin carried out mainly in South Africa was successful in 96% of carriers while only 74% success rate was recorded with vancomyin (144). In areas with high prevalence of PRSP, there is at present, no rationale for treatment of carriers, as its value in outbreak situations remains largely unproven (15).

PROBLEMS OF PRSP IN AFRICA

Little is known about the prevalence of PRSP in Nigeria and many other African countries apart from South Africa. Most health institutions in Africa lack active antimicrobial resistance surveillance, drug monitoring and infection control programmes. There is therefore apparent lack of awareness by health care providers, of the occurrence of PRSP and other resistant organisms, and their clinical significance. Added to this is the poor laboratory service in many centres, to identify and perform susceptibility testing (145).

With poor socioeconomic situations in many African countries, occasioned by bad governance, there is gross under funding of the health sector. Little attention is paid to infectious disease surveillance and control programmes, an aspect of medicine that has not been well appreciated by many authorities. Superimposed on this, is the lack of regulation on antibiotic prescription and usage. Self-medication and over-the-counter prescription of antibiotics is widespread in Nigeria and many African countries (145,146). The problem of drug resistance is therefore expected to be enormous in these countries. A limited survey in 1978 gave a PRSP prevalence rate of 20% in Nigeria (65). This has a grave implication for therapy of serious pneumococcal infection in this country. Based on this and the

available literatures from other Africa countries (10,13,61,71,98) and elsewhere (109-114), penicillin will no longer be recommended in the initial (empiric) therapy of serious pneumococcal infections in Nigeria. Ceftriaxone and cefotaxime are the preferred agents in the initial empiric therapy of pneumococcal infections of the lungs, blood stream and the central nervous system.

There is the need to increase surveillance for PRSP in health institutions and communities to determine the true prevalence and evaluate their susceptibility to newer agents. Antibiotic prescription practice should be regulated by law, with outright ban on over-the-counter sale of antibiotics. Laboratories should make available, reports of susceptibility pattern of common pathogens in the environment to the physicians and other health care providers on a regular basis. There should be a coordinated action between the various health institutions and the National Infection Control Centre, which should be responsible for storing data on susceptibility and occurrence of PRSP and other resistant organisms.

CONCLUSION

Since there is no doubt that imprudent use of antimicrobials promotes the spread of drug resistance in both the hospital and the community, the emergence of drug resistant *Streptococcus pneumoniae* is hardly surprising. Although appropriate antimicrobial use has unquestionable value, providing antimicrobials for viral infections of the upper respiratory tract does not benefit patients, it rather increases the likelihood that resistant organisms will be selected. Many instances of "presumed bacterial infection" are likely to be of viral aetiology but are misdiagnosed because of inadequate diagnostic criteria used by the physician. Physician concern over inadequate treatment for presumed bacterial infection, combined with patient pressure for prescribing antimicrobials, further complicates the problem as does the use of more expensive, broad spectrum agents, which may not be necessary unless indicated by organisms' identification and susceptibility.

The primary responsibility for identification, management and control of the spread of drug resistance pneumococcal infection lie

with the primary care physician and diagnostic laboratory. Laboratories must be equipped to isolate and perform susceptibility tests and must participate in external quality control programmes. The presence of Penicillin intermediate and resistant *Streptococcus pneumoniae,* as well as the status of other drug classes must be known in each community and updated frequently to help guide empiric therapy of infections potentially caused by these organisms since organism detection and *in vitro* testing may not be available most of the time.

REFERENCES

1. Schreiber JR, Jacobs MR. Antibiotic-resistant pneumococci. *Pediatr Clin North Am.* 1995; **42**: 519-537.
2. Poole MD. Otitis media complication and treatment failures. Implications of pneumococcal resistance. *Paediatr Infect Dis J.* 1995; **14**: S23-S26.
3. Salako LA. The challenges of infections diseases. A Nigerian perspective. *J Nig Infect Control Ass.* 1998; **1(1)**: 11-13.
4. Hansman D, Bullen MM. A resistant pneumococcus. *Lancet.* 1967; 2: 264 – 265.
5. Hansman D, Glasgow HN, Sturt J, *et al.* Pneumococci insensitive to penicillin. *Nature.* 1971; **230**: 407 – 409.
6. Dixon JMS. Pneumococcus with increased resistance to penicillin. *Lancet.* 1974; **ii**; 474.
7. Howes VJ, Mitchel RG. Meningitis due to relatively resistant pneumococcus. *Br Med J.* 1976; 1: 996.
8. Naragi S, Kirkpatrick GP, Kabin S. Relapsing pneumococcal meningitis: isolation of an organism with decreased susceptibility to Penicillin G. *J Paediatr.* 1974; **85**: 671-673.
9. Paredes A, Taber LH, Yow MD, *et al.* Prolonged pneumococcal meningitis due to an organism with increased resistance to penicillin. *Paediatrics.* 1976; **58**: 378– 361.
10. Applebaum PC, Bhamjee A, Scragg JN, *et al. Streptococcus pneumoniae* resistant to penicillin and chloramphenicol. *Lancet.* 1977; **ii**: 995 – 997.
11. Iyer PV, Kahler, JH, Jacobs NM. Penicillin resistant pneumococcal meningitis (letter). *Pediatrics.* 1978; **61**: 157 – 158.
12. Mace JW, Janik DS, Sauer RL, *et al.* Penicillin resistant pneumococcal meningitis in an immunocompromised infant (letter). *J Paediatr.* 1977; **91**: 506-507.
13. Oppenheim B, Koornhof HJ, Austrian R. Antibiotic resis-

tant pneumococcal disease in children at Baragwanath hospital, Johannesburg. *Paediatr Infect Dis J.* 1986; **5**: 520 – 524.

14. Spika JS, Facklam RR, Plikaytis BD, Oxtoby MJ, the pneumococcal surveillance working group. Antimicrobial resistance of *Streptococcus pneumoniae* in the United States 1979 – 1987. *J Infect Dis.* 1991; **163**: 1273 – 1278.
15. Klugman KP. Pneumococcal resistance to antibiotics. *Clin Microbiol Rev.* 1990; **3**: 171 – 196.
16. Johnson AP, Speller DCE, George RC, *et al.* Prevalence of antibiotic resistance in pneumococci in England and Wales: results of observational surveys in 1990 and 1995. *Br Med J.* 1996; **312**: 1454 – 1456.
17. Kanaviki S, Karabela S, Marinis E, Legakis NJ. Antibiotic resistance of clinical isolates of *Streptococcus pneumoniae* in Greece. *J Clin Microbiol.* 1994; **32**: 3056 – 3058.
18. Marchese A, Debbia EA, Arvigo A, *et al.* Susceptibility of *Streptococcus pneumoniae* strains isolated in Italy to Penicillin and ten other antibiotics. *J Antimicrob Chemother.* 1995; **36**: 833 – 837.
19. Bedess JP, Chevret S, Chastang C, *et al,* and the French Cooperative Pneumococcus Study Group. Epidemiological features of and risk factors for infection by *Streptococcus pneumoniae* strains with diminished susceptibility to penicillin. Findings of a French survey. *Clin Infect Dis.* 1996; **22**: 63 – 72.
20. Reinert RR, Queck A, kaufhold A, *et al.* Antimicrobial resistance and type distribution of *Streptococcus pneumoniae* isolates causing systemic infections in Germany 1992 – 1994. *Clin Infect Dis.* 1995; **21**: 1398 – 1401.
21. Privitera G. Penicillin resistance among *Streptococcus pneumoniae* in Europe. *Diagn Microbiol Infect Dis.* 1994, **19**. 157 – 161.
22. Linares J, Pallares R, Alonso T, *et al.* Trends in antimcrobial resistance of clinical isolates of *Streptococcus pneumoniae* in Bellvitage hospital, Barcelona, Spain (1979 – 1990). *Clin Infect Dis.* 1992; **15**: 99-105.
23. Caputo GM, Applebaum PC, Liu HH. Infections due to Penicillin Resistant Pneumococci: clinical, epidemiologic and microbiologic features. *Arch Intern Med.* 1993; **153**: 1301 – 1310.
24. Marton A. Pneumococcal antimicrobial resistance: the problem in Hungary. *Clin Infect Dis.*1992; **15**: 106-11.
25. Yoshida R, Kaku M, Kohno S, *et al.* Trends in antimicrobial resistance of *Streptococcus pneumoniae* in Japan. *An-*

timicrob Agents Chemother. 1995; **39**:1196-1198.
26. Song JH, Lee NY, Ichayama S, *et al* and the ANSORP Study Group. Spread of drug-resistant *Streptococcus pneumoniae* in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) Study. *Clin Infect Dis.* 1999; **28**: 1206-1211.
27. White B. The biology of the pneumococcus: the bacteriological, biochemical and immunologic characters and activities of *Diplococcus pneumoniae.* A Common wealth Fund Book. Copyright 1938. Cambridge MA: Reprinted by Harvard University Press. 1979.
28. Heffron R. Pneumonia, with special reference to pneumococcus lobar pneumonia. Cambridge MA. Harvard University Press. 1979.
29. Musher DM, Watson DA, Dominguez E. Pneumococcal vaccination: work to date and future perspectives. *Am J Med Sci.* 1990; **300**: 45 – 52.
30. Felton LD. Studies on the immunizing substances in pneumococci II: Separation of the organism into acid soluble and acid insoluble fractions. *J Immunol.* 1934; **27**: 397 – 393.
31. Smillie WG, Wornock GH, White HJ. A study of a type 1 pnecomoccus epidemic at the State Hospital at Worcester, Mass. *Am J Publ Health.* 1938; **28**: 293 – 302.
32. McLeod CM, Hodges RG, Heidelberger M, *et al.* Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. *J Exp Med.* 1945; **82**: 445 – 465.
33. Griffith F. The significance of pneumococcal types. *J Hyg* 1928; **27**: 113-159.
34. Hodges RG, McLeod CM, Bernhard WG. Epidemic pneumococcal pneumonia III. Pneumococcal carrier studies *Am J Hyg.* 1946; **44**: 207-230.
35. Tilgham RC, Finland M. Clinical significance of bacteraemia in pneumococcal pneumonia. *Arch Intern Med.* 1937; **59**: 602-619.
36. Austrian R, Gold J. Pneumococcal bacteraemia with especial reference to bacteraemic pneumococcal pneumonia. *Ann Intern Med.* 1964; **60**: 759-776.
37. Shapera RM, Masten JM. Host factors and capsular typing of body fluids isolates in fulminant pneumococcal infections. *Infect Immunol.* 1972; **5**: 132-136.
38. Ritcher RW, Brust JCM. Pneumococcal meningitis in Harlem Hospital. *NY State J Med.* 1971; **71**:2747-2754.
39. Mufson MA, Kruss DM, Wasil RE, Metzger WI. Capsular types and outcome of bacteraemia pneumococcal dis-

ease in the antibiotic era. *Arch Intern Med.* 1974; **134**:505-510.
40. Eriksen KR. Studies of induced resistance to penicillin in a pneumococcus type I. *Acta Pathol. Microbiol Scand.* 1945; **22**: 398-405.
41. Evan W, Hansman D. Tetracycline resistant pneumococcus. *Lancet.*1963; **i**: 451
42. Turner GC: Tetracycline resistance pneumococci in a general hospital. *Lancet.* 1963; **2**: 1292-1295.
43. Dixon JMS. Pneumococcus resistance to erythromycin and lincomycin. *Lancet.* 1967; **1**: 573.
44. Kislak JW. Type 6 pneumococcus resistant to erythromycin and lincomycin. *N Engl J Med.* 1967; **276**: 852.
45. Morgenroth J, Karfman M. Arzneifestigkeit bei Bakterien (Pneumokokken) *Z. Immunitacts forsch.* 1912; **15**: 610-624.
46. Moore HF, Chesney AM. A study of ethylhdrocupreine (Optochin) in the treatment of acute lobar pneumonia. *Arch Intern Med.* 1917; **19**: 611.
47. Frisch AW, Price AE, Myers GB. Type VIII pneumococcus: Development of sulfadiazine resistance, transmission by cross infection and persistence in carriers. *Ann Intern Med.* 1943; **18**: 271-278.
48. Kislak JW, Razavi LMB, Dlay AK, *et al.* Susceptibility of pneumococci to nine antibiotics. *Am J Med Sci.* 1965; **250**: 261-268.
49. National Committee for Clinical Laboratory Standard. Performance standard for antimicrobial susceptibility testing. Sixth informational supplement. NCCLS document M100-S6, Wayne, PA: National Committee for Clinical Laboratory Standards. 1995: 15(14).
50. National Committee for Clinical Laboratory Standards. Performance standard for antimicrobial susceptibility testing. Fifth informational supplement M100-S5. NCCLS. Villanova Pa, 1994.
51. Lederberg J, Shope RE, Oaks SC Jr (eds). Emerging infections: microbiologic threats to health in the United States. Washington DC: National Academy Press. 1992.
52. Thornsberry C, Brown SD, Yee C, *et al.* Increasing penicillin resistance in *Streptococcus pneumoniae* in the United States. *Infect Med.* 1993; **93**: 1-24.
53. Barry AL, Pfaller MA, Fuchs PC, *et al. In vitro* activities of 12 orally administered antimicrobial agents against four species of bacterial respiratory pathogens from US medical centres in 1992 and 1993. *Antimicrob Agents Chemother.* 1994; **38**: 2419-2425.

54. Chesney PJ. The escalating problem of antimicrobial resistance in *Streptococcus pneumoniae*. *Am J Dis Child.* 1992; **146**: 912-916.
55. Butler JC. Breiman RF, Facklam RR, the Pneumococcal Working Group. Emergence of drug resistant pneumococci in the United States. In: Program and abstracts of the 33rd Inter Science Conference on antimicrobial agents and chemotherapy. Washington DC. American Society for Microbiology. 1993: 336.
56. Centre for Disease Control and Prevention (CDC). Drug resistant *Streptococcus pneumoniae* in Kentucky and Tennessee. 1993. *Morb Mortal Wkly Rep.* 1994; **43**: 23-31.
57. Doern GV, Brueggemann A, Holley HP Jr, *et al.* Antimicrobial resistance of *Streptococcus pneumoniae* recovered from outpatients in the United States during the winter months of 1994 to 1995; results of a 30-centre national surveillance study. *Antimicrob. Agents Chemother.* 1996; **40**: 1208-1213.
58. Henderson FW, Gilligan PH, Wait K, *et al.* Nasopharyngeal carriage of antibiotic resistant pneumococci by children in group day care. *J Infect Dis.* 1988; **157**: 256-263.
59. Doyle MG, Morrow AL, Van R, *et al.* Penicillin Resistant *Streptococcus pneumoniae* in children in home care and day care. *Paediatr Infect Dis J.* 1992; **11**: 831-835.
60. Jacobs MR, Koornhof HJ, Robins-Browne RM, *et al.* Emergence of multiply resistant pneumococci. *N Engl J Med.* 1978; **299**: 735-740
61. Wamola [illegible] Mirza NB, Nsnazumuhure H. Penicillin Resistant Pneumococcal meningitis in Kenyatta National Hospital Nairobi, Kenya. *East Afr Med J.* 1981; **58**: 12-17.
62. Kell CM, Jordens JZ, Daniels M, *et al.* Molecular epidemiology of penicillin- resistant pneumococci isolated in Nairobi, Kenya. *Infect Immunol.* 1993; **61(10)**: 4382-4391.
63. Baquero F, Loza E. Geography and genetics of penicillin resistance. In: Klugman K (ed.). Pneumococcus with particular reference to Penicillin Resistant *Streptococcus pneumoniae*, Part I. 1994: 27-32.
64. Fredriksen B, Henrichsen J. Throat carriage of *Streptococcus pneumoniae* and *Streptococcus pyogenes* among infants in Zambia. *J Trop Paediatr.* 1988; **34**: 114 – 117.
65. Hansman D. Chloramphenicol resistant pneumococci in West Africa (letter). *Lancet.* 1978; **i**: 1102 – 1103.
66. Moreno S, Garcia-Leoni ME, Cercenado E, *et al.* Infections caused by Erythromycin-Resistant *Streptococcus pneumoniae*, incidence, risk fac-

tors and response to therapy in a prospective study. *Clin Infect Dis.* 1995; **20**: 1195 – 1200.

67. Bradley JS, Connor JD. Ceftriaxone failure in meningitis caused by *Streptococcus pneumoniae* with reduced susceptibility to β-lactam antibiotics. *Paediatr Infect Dis J.* 1991; **10**: 871-873.
68. Sloas MM, Barrett FF, Chesney PJ, *et al.* Cephalosporin treatment failure in Penicillin and Cephalosporin Resistant *Streptococcus pneumoniae* meningitis. *Paediatr Infect Dis J.* 1992; **11**: 662-666.
69. Tenover FC, Swenson JM, McDougal LK. Screening for extended spectrum cephalosporin resistance in pneumococci. *Lancet.* 1992; **340**: 1420.
70. Linares J, Alonso T, Perez JL, *et al.* Decreased susceptibility of Penicillin Resistant Pneumococcus to 24 β-lactam antibiotics. *J Antimicrob Chemother* 1992; **30**: 279-288.
71. Klugman KP, Saunders J. Pneumococci resistant to extended spectrum cephalosporins in South Africa. *Lancet.* 1993; **341**: 1164.
72. Mason EO, Kaplan SL, Lamberth LB, *et al.* Increased rate of isolation of Penicillin Resistant *Streptococcus pneumoniae* in a children's hospital and *in vitro* susceptibility to antibiotics of potential therapeutic use. *Antimicrob Agents Chemother.* 1992; **36**: 1703-1707.
73. Welby PL, Keller DS, Cromien JL, *et al.* Resistance to Penicillin and non β-lactam antibiotics of *Streptococcus pneumoniae* at a children's hospital. *Paediatr Infect Dis J.* 1994; **13**: 281-287.
74. Lonks JR, Medeiros AA. High rate of erythromycin and clarithromycin resistance among *Streptococcus pneumoniae* isolates from blood cultures from Providence RI. *Antimicrob Agents Chemother.* 1993; **37**: 1742-1745.
75. Munoz R, Musser JM, Crain M, *et al.* Geographic distribution of penicillin resistant clones of *Streptococcus pneumoniae:* characterization by penicillin binding protein profile, surface protein A typing and multilocus enzyme analysis. *Clin Infect Dis.* 1992; **15**: 112-118.
76. McGee L, McDougal L, Zhou J, *et al.* Nomenclature of Major Antimicrobial-Resistant Clones of *Streptococcus pneumoniae* Defined by the Pneumococcal Molecular Epidemiology Network. *J Clin Microbiol.* 2001; **39(7)**: 2565-2571.
77. Zhou J, Enright MC, Spratt BG. Identification of the major Spanish clones of penicillin-resistant pneumococci via the Internet using multilocus sequence typing. *J Clin Microbiol.* 2000; **38**: 977-986.
78. Munoz R, Coffey TJ, Daniels

M, *et al.* Intercontinental spread of a multiresistant clone of serotype 23F *Streptococcus pneumoniae. J Infect Dis.* 1991; **164**: 302-306.

79. Soares S, Kristinsson KG, Musser JM, Tomasz A. Evidence for the introduction of a multiresistant clone of serotype 6B *Streptococcus pneumoniae* from Spain to Iceland in the late 1980s. *J Infect Dis.* 1993; **168**: 158-163.
80. Reichmann P, Varon E, Gunther E, *et al.* Penicillin-resistant *Streptococcus pneumoniae* in Germany: genetic relationship to clones from other European countries. *J Med Microbiol.* 1995; **43**: 377-385.
81. Lefevre JC, Bertrand MA, Faucon G. Molecular analysis by pulsed-field gel electrophoresis of penicillin-resistant *Streptococcus pneumoniae* from Toulouse, France. *Eur J Clin Microbiol Infect Dis.* 1995; **14**: 491-497.
82. Ip M, Lyon DJ, Yung RWH, *et al.* Introduction of new clones of Penicillin-Non susceptible *Streptococcus pneumoniae* in Hong Kong. *J Clin Microbiol.* 2002; **40(4)**: 1522-1525.
83. Tomasz A. Biochemistry and genetics of penicillin resistance in pneumococci. In: Ferrtti JJ, Curtis R III (eds.). Streptococcal genetics. Washington DC. American Society for Microbiology. 1987: 87-92.
84. Elderbroth H, Hackenbeck R. Penicillin degrading activities of peptides from Pneumococcal Penicillin Binding Protein. *Eur J Biochem.* 1988; **171**: 219-224.
85. Markiewicz Z, Tomasz A. Variation in Penicillin Binding Protein pattern of penicillin resistant clinical isolates of pneumococci. *J Clin Microbiol.* 1989; **27**: 405-410.
86. Hackbath CJ, Chambers HF. Methicillin resistant staphylococci. Genetic and mechanism of resistance. *Antimicrob Agents Chemother.* 1989; **33**: 991-994.
87. Hackenbeck R, Tarpay M, Tomasz A. Multiple changes of Penicillin Binding Proteins in penicillin resistant clinical isolates of *Streptococcus pneumoniae. Antimicrob Agents Chemother.* 1980; **17**: 364-371.
88. Dowson CG, Hutchison A, Brannigan JA, *et al.* Horizontal transfer of PBP genes in penicillin resistant clinical isolates of *Streptococcus pneumoniae. Proct. Natl. Acad. Sci. USA.* 1989; **86**: 8842-8846.
89. Courvalin P, Carlier C. Transposable multiple antibiotic resistance in *Streptococcus pneumoniae. Mol Gen Genet.* 1986; **205**: 291-297.
90. Santagati M, Iannelli F, Oggioni MR, *et al.* Characterization of a genetic element carrying the macrolide efflux gene *mef* (A) in *Streptococcus pneumoniae. Antimicrob Agent*

Chemother. 2000; **44**: 2585-2587.

91. Leclercq R, Courvalin P. Bacterial resistance to macrolide, lincosamide and streptogamin antibiotics by target modification. *Antimicrob Agents Chemother.* 1991; **35**: 1267-1272.
92. Clewell DB, Flamagan SE, Jaworski DD. Unconstrained bacterial promiscuity; the Tn *916*- Tn *1545* family of conjugative transposons. *Trends Microbiol.* 1995; **3**: 229-236.
93. Adrian PV, Klugman KP. Mutations in the dihydrofolate reductase gene of trimethoprim-resistant isolates of *Streptococcus pneumoniae. Antimicrob Agents Chemother.* 1997; **41**: 2406-2413.
94. Dang-Van A, Triaby G, Acar JF, *et al.* Chloramphenicol resistance in *Streptococcus pneumoniae*: Enzymatic acetylation and possible plasmid linkage. *Antimicrob Agents Chemother.* 1978; **13**: 557-583.
95. Hooper DC, Wolfson JS, Fluoroquinolone antimicrobial agents. *N Engl J Med.* 1991: **324**: 384-394.
96. Gootz TD, Zanfwwski R, Haskell S, *et al.* Activity of the new fluoroquinolone trovafloxacin (C-99, 219) against DNA gyrase and topoisomerase IV mutants of *Streptococcus pneumoniae* selected *in vitro. Antimicrob Agents Chemother.* 1996; **40**: 2691-2697.
97. Jernigan DB, Cetron MS, Breiman RF. Defining the public health impact of drug resistant *Streptococcus pneumoniae*: report of a working group. MMWR *Morb Mortal Wkly Rep.* 1996; **45**: 1-20.
98. Jacobs MR. Treatment and diagnosis of infectious diseases caused by drug resistant *Streptococcus pneumoniae. Clin Infect Dis.* 1992; **15**: 119-127.
99. Waitz JA. Performance standards for antimicrobial susceptibility testing. National Committee for Clinical Laboratory Standards. Pa. 1986; **6**: 21.
100. Swenson JM, Hill BC, Thornsberry C. Screening pneumococci for penicillin resistance. *J Clin Microbiol.* 1986; **24**: 749-752.
101. Ragsdale AR, Standord JP. Interfering effect of incubation in Carbon dioxide (CO_2) on the identification of pneumococci by optochin disc. *Appl Microbiol.* 1971; **22**: 854-855.
102. Kontiainen S, Sivonen A. Optochin resistance in *Streptococcus pneumoniae* strains isolated from blood and middle ear fluids. *Eur J Clin Microbol.* 1987; **6**: 426-424.
103. Munoz R, Fenoll A, Vicioso D, *et al.* Optochin resistance variants of *Streptococcus pneumoniae. Diag Microbol Infect Dis.* 1990; **13**: 63-66.

104. Waites KB, Swiatlo E, Gray BM. Comparative activities of parenteral cephalosporins against Penicillin Resistant *Streptococcus pneumoniae* isolated from paediatric patients. *Curr Ther Res.* 1996; **57**: 489-496.
105. American Academy of Paediatrics Committee on Infectious Diseases. Therapy for children with invasive pneumococcal infections. *Paediatrics.* 1997; **99**: 289-299.
106. D'Amato RF, Swenson JA, McKinley GA, *et al.* Quantitative antimicrobial susceptibility test for *Streptococcus pneumoniae*, using inoculum supplemented with whole defibrinated sheep blood. *J Clin. Microbiol.* 1987; **25**: 1753-1756,
107. Jorgensen JH, Howell AW, Maher LA. Quantitative antimicrobial susceptibility testing of *Haemophilus influenzae* and *Streptococcus pneumoniae* by using the E-test. *J Clin Microbol.* 1991; **29**: 109-114.
108. Marcias EA, Mason EO, Ocera HY, La Rocco MT. Comparison of E-test with standard microdilution for determining antibiotic susceptibility of penicillin-resistant strains of *Streptococcus pneumoniae. J Clin Microbiol.* 1994; **32**: 430-432.
109. Friedland TR, McCracken GH Jr. Management of infections caused by antibiotic resistant *Streptococcus pneumoniae. N Engl J Med.* 1994; **331**: 337-382.
110. Klugman KP. Epidemiology, control and treatment of multidrug resistant pneumococci. *Drugs.* 1996; **52** (Suppl. 2): 42-46.
111. Bradley JS, Kaplan SL, Klugman KP, *et al.* Consensus management of infections in children caused by *Streptococcus pneumoniae* with decreased susceptibility to penicillin. *Paediatr Infect Dis J.* 1995; **14**: 1037-1041.
112. Rubinstein E, Rubinovitch B. Treatment of severe infections caused by Penicillin Resistant Pneumococci. Role of third generation cephalosporins. *Infection.* 1994; **22** (Suppl. 3): S116-166.
113. Leggiardro RJ. Penicillin and cephalosporin resistant *Streptococcus pneumoniae*, an emerging antimicrobial threat. *Paediatrics.* 1994; **93**: 500-503.
114. Viladrich PF, Gudiol F, Linares J, *et al.* Characteristics and antibiotic therapy of adult meningitis due to Penicillin Resistant Pneumococci. *Am J Med.* 1988; **84**: 839-846.
115. Viladrich PF, Gudiol, Linares J, *et al.* Evaluation of vancomycin for therapy of adult pneumococcal meningi-

tis? *Antimicrob Agents Chemother.* 1991; **35**: 2467-2472.
116. Friedland IR, Paris MM, Ehrett S, *et al.* Evaluation of antimicrobial regimens for treatment of experimental penicillin and cephalosporin resistant pneumococcal meningitis. *Antimicrob Agents Chemother.* 1993; **37**: 1630-1636.
117. Paris MM, Hickey SM, Uscher MI, *et al.* Effect of dexamethasone therapy on experimental penicillin and cephalosporin resistant pneumococcal meningitis. *Antimicrob Agents Chemother.* 1994; **38**: 1320-1344.
118. Klugman KP, Friedland IR, Bradley IS. Bactericidal activity against cephalosporin resistant *Streptococcus pneumoniae* in cerebrospinal fluids of children with acute bacterial meningitis. *Antimicrob Agents Chemother.* 1995; **39**: 1988-1992.
119. Friedland IR, Klugman KP. Failure of chloramphenicol therapy in Penicillin Resistant Pneumococcal meningitis. *Lancet.* 1992; **339**: 405-408.
120. Friedland IR, Shelton S. McCracken GH Jr. Chloramphenicol in penicillin resistant pneumococcal meningitis. *Lancet.* 1993; **342**: 240-241
121. Jacobs MR. Increasing importance of antibiotic resistant *Streptococcus pneumoniae* in Acute Otitis M[illegible] *Paediatr Infect Dis J.* 1[illegible] **15**: 940-943.
122. Block SL, Harrison CJ, Hedrick JA, *et al.* Penicillin Resistant *Streptococcus pneumoniae* in Acute Otitis Media: risk factors, susceptibility patterns and antimicrobial management. *Paediatr Infect Dis J.* 1995; **14**: 751-759.
123. Hoberman A, Paradise J. Blocks S, *et al.* Efficacy of Amoxicillin/Clavulanate for Acute Otitis Media: Relation to *Streptococcus pneumoniae* susceptibility. *Paediatr Infect Dis J.* 1996; **15**: 955-962.
124. Dagan R, Abramson O, Leibovitz E, *et al.* Impaired bacteriologic response to oral cephalosporins in Acute Otitis Media caused by pneumococci with intermediate resistance to penicillin. *Paediatr Infect Dis J.* 1996; **15**: 980-985.
125. Barry B, Gehanno P, Blumen M, *et al.* Clinical outcome of Acute Otitis Media caused by pneumococci with decreased susceptibility to penicillin. *Scand J Infect Dis.* 1994; **26**: 446-452.
126. Gehanno P, Lenoir G, Berche P. *In vivo* correlates for *Streptococcus pneumoniae* penicillin resistance in Acute Otitis Media. *Antimicrob Agents Chemother.* 1995; **39**: 271-272.
127. Pallares R, Linares J, Vadillo M, *et al.* Resistance to penicillin and cephalosporin

and mortality from severe pneumococcal pneumonia in Barcelona, Spain. *N Engl J Med.* 1995; **333**: 474-480.

128. American Thoracic Society. Guidelines for initial management of adults with community acquired pneumonia: diagnosis, assessment of severity and initial antimicrobial therapy. *Am Rev Respir Dis.* 1993; **148**: 1418-1420.
129. Feldman C, Klugman KP. Antibiotic resistant pneumococcal pneumonia. *S Afr Med J.* 1996; **86**: 28-30.
130. Friedland R. Comparison of the response to antimicrobial therapy of penicillin resistant and penicillin susceptible pneumococcal disease. *Paediatr Infect Dis J.* 1995: **14**: 885-890.
131. Feikin DR, Schuchat A, kolazak M, *et al.* Mortality from invasive pneumococcal pneumonia in the era of antibiotic resistance, 1995-1997. *Am J Publ Health.* 2000; **90 (2)**: 223-229.
132. Weis AJ, Klesper ME, Ernst EJ, *et al.* Evaluation of the costs associated with the treatment of *Streptococcus pneumoniae:* penicillin susceptible and resistant, from 1992-1996 at a University Teaching Hospital. Paper presented at the American College of Clinical Pharmacy, Annual meeting, Phoenix AZ; 1997.
133. Cates KL, Gerrad JM, Giebink GSS, *et al.* A Penicillin Resistant Pneumococcus. *J Paediatr.* 1978; **93**: 624-626.
134. Bolan G, Broome CV, Facklam RR, *et al.* Pneumococcal vaccine efficacy in selected population in the United States. *Ann Intern Med.* 1986; **104**: 1-6.
135. Butter JC, Breiman RF, Lipman HB, *et al.* Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978 – 1994. Implication for development of a conjugate vaccine. *Infect Dis.* 1995; **171**: 885-889.
136. Robbins JB, Austrian R, Lee CJ, *et al.* Consideration for formulating the second generation pneumococcal capsular polysaccharide vaccine with emphasis on cross-reactive types within groups *J Infect Dis.* 1983; 1136 – 1159.
137. Robbins JB, Lee CJ, Rastogi SC, *et al.* Comparative immunogenicity of group 6 pneumococcal type 6A (6) and type 6B(26) capsular polysaccharides. *Infect Immunol.* 1979; **26**: 1116-1122.
138. Advisory Committee on Immunization Practices (ACIP). Pneumococcal polysaccharide vaccine. MMWR *Morb Mortal Wkly Rep.* 1989; **38**: 64-68, 73-76.
139. Centre for Disease Control and Prevention (CDC). Pre-

vention of pneumococcal disease: recommendation of the Advisory Committee on Immunization Practice (ACIP). MMWR *Morb Mortal Wkly Rep.* 1997; **46**(RR-08): 1-24.

140. Kayhty H, Ahman H, Romberg PR, *et al.* Pneumococcal polysaccharide - meningococcal outer membrane protein complex conjugate vaccine is immunogenic in infants and children. *J Infect Dis.* 1995; **172**: 1273 – 1278.
141. Leach A, Ceesay SJ, Banya WAS, *et al.* Pilot trial of a pentavalent pneumococcal polysaccharide/protein conjugate vaccine in Gambian infants. *Paediatr Infect Dis J.* 1996; **15**: 33-339.
142. Centre for Disease Control and Prevention: Preventing pneumococcal disease among infants and young children: recommendation of the Advisory Committee on Immunization Practice (ACIP). MMWR *Morb Mortal Wkly Rep.* 2000; **49** (RR-09): 1-35.
143. Pinner RW, Rebmann CA, Schuchat A, Hughes JM. Disease surveillance and the academic, clinical and public health communities. *Emerg Infect Dis.* 2003; **9(7)**: 781-787.
144. Koornhof HJ, Jacobs MR, Ward JI, *et al.* Therapy and control of antibiotic resistant pneumococcal disease. In: D. Schlessinger (ed.). Microbiology. Washington DC. American Society for Microbiology. 1979: 286 – 289.
145. Okeke IN, Sosa A. Antibiotic resistance in Africa; discerning the enemy and plotting a defence. *Africa Health.* 2003; **25 (3)**: 10-15.
146. Odugbemi T. The use and misuse of antibiotics. *Nig Med Pract.* 1981; **1**: 4-8.