INVASIVE DISEASE CAUSED BY *STREPTOCOCCUS PNEUMONIAE* RESISTANT TO PENICILLIN AND THIRD-GENERATION CEPHALOSPORINS

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DECLARATION

I, Ranmini Sumudita Kularatne, declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

....(Signature)

....16th.....day of...December, 1999.

DEDICATION

This research report is dedicated to my mother, whose encouragement, support and guidance throughout the years has been invaluable.

ABSTRACT

Resistance to penicillin G and third-generation cephalosporins among *Streptococcus pneumoniae* is an emerging and increasing microbial threat. Over a 4½ year study period, we identified 40 patients with either intermediate (26/40, 65%) or high-level (14/40, 35%) resistance to third-generation cephalosporins (i.e., ceftriaxone and cefotaxime). Fifteen subjects were adults (mean age 47 years) and 25 were children (mean age 26.5 months). Study isolates were recovered from the following body sites: blood (25 isolates); CSF (5); sputum (5); tracheal aspirates (4); eye socket swabs (4); bronchial washings (1); and ascitic fluid (1). Sites of primary pneumococcal infection were: pneumonia (26 cases); meningitis (5); skin and soft tissue (post-enucleation for retinoblastoma)(4); primary bacteremia (4); and primary peritonitis (1). Sixty-three percent of infections were community-acquired and 37% were nosocomial in origin. Thirty-three percent and 29% of subjects with community-acquired and nosocomial infection, respectively, had been hospitalized within the three months prior to pneumococcal infection. Overall, one third of patients had received prior antibiotic therapy. HIV/AIDS was the most common underlying condition for both adults and children. Overall mortality was 10.5% (4/38 patients), all were female adults with poor prognostic features including severe underlying illness, advanced age, nosocomial pneumococcal infection, and multilobar pneumonia. Of the three patients with both clinical and microbiological evidence of meningitis, two received appropriate combination therapy and recovered; one patient receiving ceftriaxone alone died. All study isolates were contained within the current 23-polyvalent vaccine, and 84% of patients were vaccine candidates. More accurate surveillance of cephalosporin resistance among pneumococci is needed including routine screening of clinically significant isolates. Modification of current empiric treatment guidelines for suspected pneumococcal meningitis may be necessary based upon local, regional, and national prevalences of cephalosporin resistance.

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CHAPTER 1

INTRODUCTION

In the new millennium, *Streptococcus pneumoniae* remains one of the most common and important causes of invasive bacterial disease in the world. Despite the availability of an effective vaccine, pneumococcal otitis media in children and the more lethal forms of pneumonia, meningitis, and primary bacteremia affect millions of individuals and claim hundreds of thousands of lives around the globe. Further highlighting its seriousness as a worldwide pathogen is the rising incidence of resistance of *Streptococcus pneumoniae* to penicillin and other antibiotics in many geographic areas.

Resistance to third-generation cephalosporins among *Streptococcus pneumoniae* is an emerging and increasing microbial threat [1-5]. Such resistance correlates with the increasing prevalence of penicillin-resistant strains of pneumococci, and presents a critical challenge to clinicians in managing patients with invasive pneumococcal disease, especially pneumococcal meningitis, resistant to these agents. For non-critically ill patients with non-meningeal pneumococcal infection, such as pneumonia caused by these strains, therapy with penicillin G and other active β-lactam antibiotics is generally effective [3,6-9], and is explained by achievable serum concentrations that greatly exceed the MICs of these resistant strains. However, penicillin G is inadequate for meningitis caused by both intermediate- and high-level penicillin-resistant strains of pneumococci because the levels achieved in the CSF have inadequate bactericidal activity against these organisms. Third-generation cephalosporins have been considered the therapeutic agents of choice in pneumococcal meningitis caused by these strains because their MICs are usually 2 to 4 times lower than those for penicillin G and they achieve high serum levels and have excellent penetration into the CSF.

Although South Africa has one of the highest rates of multiply drug resistant pneumococcal disease in the world, no case series have previously been published dealing with clinically significant cephalosporin resistance. In this report, we review the epidemiology and microbiologic and clinical features or 40 episodes of invasive pneumococcal infection caused by *Streptococcus pneumoniae* resistant to penicillin G and third-generation cephalosporins in a South African population. The objectives of the study were to determine the epidemiology and incidence; the demographic characteristics; the microbiological and clinical features and the clinical outcome of invasive drug-resistant pneumococcal infection. Another aim was to establish whether the currently available 23 polyvalent pneumococcal vaccine is appropriate for use in our region, based on the prevalence of local pneumococcal serotypes.

CHAPTER 2

MATERIALS AND METHODS

From January 1995 through July 1999, this retrospective study was conducted at the Chris Hani Baragwanath Hospital, a 3,300-bed public hospital located in the former black township of Soweto), and major academic teaching affiliate of the University of the Witwatersrand in Johannesburg, South Africa. Computerized logbooks of Clinical Microbiology Laboratory were retrospectively reviewed in order to identify clinical isolates of *Streptococcus pneumoniae* that were resistant to third-generation cephalosporins. According to National Committee for Clinical Laboratory Standards [10] intermediate and high-level antibiotic resistance was defined as follows: a MIC for penicillin G 0.12-1.0 μg/ml and ≥ 2 μg/ml, respectively; cefotaxime 1 μg/ml and ≥ 2 μg/ml, respectively; and ceftriaxone 1 μg/ml and ≥ 2 μg/ml, respectively. For patients identified with infections caused by these isolates, a medical record review was performed in order to determine the following demographic, clinical, and laboratory characteristics (age; sex; HIV antibody status; CD4+ lymphocyte count; presence of underlying diseases; peripheral white blood cell count; clinical site of isolation of the study organism; site of acquisition of the study isolate (community-acquired or nosocomial); previous hospitalization (within 3 months of isolation of the study organism); prior antibiotics and clinical course (also within 3 months of isolation of the study organism); *in vitro* susceptibility pattern of the study isolate including minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for penicillin G and ceftriaxone; pneumococcal serotype; treatment regimen for the infection(s) caused by the study isolate; and clinical outcome.

Antibiotic susceptibility testing was performed using a standard disk diffusion method [11]. Minimal inhibitory concentrations (MICs) were determined only for pneumococcal isolates exhibiting penicillin resistance on disk diffusion testing and utilized a standard microdilution method in Mueller-Hinton broth supplemented with Ca^{++} and Mg^{++}. Minimal bactericidal concentrations (MBCs) were determined by spread-plating onto sheep blood agar 100 µl from the MIC assay microdilution tubes that had no visible bacterial growth and observing for colony formation. Antibiotics tested included penicillin G, ceftriaxone, cefotaxime, cefuroxime, erythromycin, chloramphenicol, clindamycin, tetracycline, trimethoprim-sulfamethoxazole, rifampin, ofloxacin, and vancomycin.

Statistical analysis of data was performed using the chi square method on an EpiInfo Version 6.02 computer software program.

CHAPTER 3

RESULTS

During the 4½ year study period, a total of 40 patients were identified with either intermediate (26/40, 65%) or high-level (14/40, 35%) resistance to third-generation cephalosporins (i.e., ceftriaxone and cefotaxime). Complete microbiologic and clinical data was available for 38 subjects (Tables 1-6). The male:female ratio was 22:18. Fifteen subjects were adults and 25 were children. For adults, the mean age was 47 years (range 22-76 years); 6 (40%) were aged ≥ 60 years. For children, the mean age was 26.5 months (range 3 months-135 months). Where patient age was known, all but one child were below the age of 5 years; 9 (39%) were between 2 and 5 years of age; and 13 (57%) were below age 2 years.

Cephalosporin-resistant pneumococcal isolates were recovered from the following body sites: blood (25 isolates); CSF (5); sputum (5); tracheal aspirates (4); eye socket swabs (4); bronchial washings (1); and ascitic fluid (1). Sites of primary pneumococcal infection were as follows: pneumonia (26 cases); meningitis (5); skin and soft tissue (post-enucleation for retinoblastoma)(4); primary bacteremia (4); and primary peritonitis (1). Some patients (all children) had concomitant otitis media (8 patients) or exudative tonsillitis (1).

Twenty-four patients (63%) were found to have community-acquired pneumococcal infection, while 14 (37%) had infections that were believed to be nosocomial in origin. For hospital-acquired infection the mean duration of hospital stay prior to pneumococcal infection was 71 days. Eight of 24 (33%) subjects with community-acquired infection had been hospitalized for any reason within the three months prior to pneumococcal infection; and four of 14 (29%) with nosocomial infection had also been previously hospitalized. The use of antibiotics within the previous three months was also analyzed; overall, 13 patients (34%) had received prior known antibiotic therapy and an additional 3 (8%) had received antimicrobial treatment during a previous hospitalization for infection although the specific agents were not known. Prior antibiotics for six patients (25%) with community-acquired infection included rifampin (3 patients); TMP-SMX (3); ampicillin (2); amoxicillin (1); amoxicillin-clavulanate (1); cefuroxime (1); cephalexin (1); and gentamicin (1), while antimicrobials for seven patients (50%)(p = 0.16) with nosocomial infection included rifampin (3 patients); erythromycin (2); TMP-SMX (2); and amoxicillin,

ceftriaxone, cefuroxime, cloxacillin, topical chloramphenicol, and topical tetracycline (1 each).

Table 6 shows underlying diseases that were present for the study cohort. HIV/AIDS was the most common underlying condition for both adults and children. Ten children were HIV-seropositive, six were HIV-negative, and in nine cases HIV antibody status was unknown. Six out of the seven (86%) malnourished children were also HIV-seropositive. Seven adults were HIV-infected, one was HIV-seronegative, and HIV antibody status was undetermined in seven. The CD4+ lymphocyte count was known for only two children, representing C1 and C2 stages, respectively [12]. For adults, CD4+ count was known for five patients (mean, 154 cells/mm^3: range, 1-360 cells/mm^3). Nine out of the ten HIV-infected children were below 2 years of age (mean, 10.8 months) and all HIV-seropositive adults except one were below 50 years of age (mean, 31.3 years).

Leukocytosis was present for 24 of 38 (63 %) of study patients with invasive cephalosporin-resistant *Streptococcus pneumoniae* infection (mean WBC count, 24,400/mm^3). Four patients (all with malignancy and undergoing cytotoxic chemotherapy) had leukopenia (mean WBC count, 1.9/ mm^3). For the 26 patients with pneumonia, chest radiographs were performed in 25 instances ; chest x-ray patterns were as follows: bilateral bronchopneumonia or consolidation - 20 patients; unilateral consolidation (unilobar or multilobar) - 6 patients; pleural effusions - 2 patients; hilar and paratracheal adenopathy - 1 patient. Of the ten patients whose clinical isolates was recovered from pulmonary secretions, 8 had co-pathogens. In addition to *S. pneumoniae*,

other organisms recovered in patients with polymicrobial pneumonia were *H. influenzae* type B (3), *Klebsiella* spp. (2), methicillin-resistant *Staphylococcus aureus* (2), *Escherichia coli* (2), *Pseudomonas aeruginosa* (1), *Enterobacter* spp. (1), *Proteus mirabilis* (1), *H. parainfluenzae* (1), *Candida glabrata* plus, *C. albicans* (1).

Of the four evaluable patients with *Streptococcus pneumoniae* recovered from CSF in our study, only one received optimal combination therapy with vancomycin plus ceftriaxone, and a clinical response was observed by the second day of treatment. Two others patients were treated with ceftriaxone: one died after two days of treatment and the other exhibited a clinical response only after 6 days of antibiotic therapy. Of note, this latter patient also received rifampin (to which the organism was susceptible) for the first four days as part of empiric antituberculous therapy. All four of these pneumococcal isolates were serotype 23, and all had MICs showing intermediate resistance to both ceftriaxone and cefotaxime. The fourth pneumococcal strain isolated from the CSF, was in a one-year-old infant with hemolytic anaemia, bronchopneumonia, and severe bilateral otitis media. Microscopic and biochemical examinations of CSF were normal, but *S pneumoniae* serotype 23 was isolated from culture. Minimal inhibitory concentration testing showed high-level resistance to ceftriaxone. There was no concomitant bacteremia, the patient remained afebrile throughout his hospital stay, and his clinical features were not compatible with a diagnosis of meningitis. Samples of CSF obtained at 24, 48 and 72 hours were all sterile. The patient received only oral erythromycin and cephalexin to which his organism showed *in-vitro* resistance, yet he recovered and in all likelihood the infecting organism represented contamination.

Four of the 38 (10.5%) evaluable patients (all adult females) died. Two of these were patients with HIV/AIDS who developed severe nosocomial pneumonia while receiving antituberculous therapy at a chronic care facility. Two persons, one with multiple myeloma and primary bacteremia, and the other with Castleman's disease (post-splenectomy) and pneumococcal meningitis, also died. Two fatal cases were infected with pneumococcal serotype 19, and two with serotype 23. All four patients who died (patients 3,11,31, and 40) had received inappropriate antibiotic therapy for their invasive pneumococcal infection according to *in vitro* susceptibility testing.

CHAPTER 4

DISCUSSION

Third-generation cephalosporins have an important role in the treatment of penicillin-resistant invasive pneumococcal disease, especially meningitis. The incidence of resistance to agents such as ceftriaxone and cefotaxime is associated with penicillin-resistance, but fortunately has been low in most areas. In a study of 462 episodes of pneumococcal bacteremia in a population with high HIV seroprevalence in New York City, Turett and colleagues documented penicillin resistance in 17% of isolates and high-level resistance (i.e., a minimal inhibitory concentration of > 1 μg/ml) in 65% of strains, yet only one penicillin-resistant isolate was also resistant to ceftriaxone and cefotaxime, both at intermediate levels [13]. In South Africa, the proportion of penicillin-resistant pneumococci with cefotaxime and/or ceftriaxone MICs of ≥ 0.5 ug/ml increased from 0% in 1986 to 21% in 1989/91 [6,14]. Strains of *S. pneumoniae* in blood and CSF demonstrating high-level resistance (MIC > 2 μg/ml) to penicillin G are relatively rare in South Africa; with a prevalence rate of 2.3% during 1987 to 1990 [15]. Thirty percent of fully penicillin-resistant pneumococci have ceftriaxone/cefotaxime MICs ≥ 2 μg/ml [16], and from this data one can estimate that high-level ceftriaxone/cefotaxime resistance is present in < 1% of all blood and CSF isolates [16]. In the present 1995-1999 analysis of blood and CSF isolates with both high- and low-level resistance, prevalence rates were 30% and 1.4% for penicillin G and third-generation cephalosporins, respectively.

Our results confirm that there is a close correlation between MICs of penicillin G and third- generation cephalosporins [2,3,17,18]. Over time, the MICs of penicillin G and third-

generation cephalosporins against strains of pneumococci began to converge: in 1995, the MICs of penicillin G were usually 2 to 4 times higher than those of cefotaxime or ceftriaxone, while in 1997-1998 MICs were generally comparable or those for penicillin G only 1 to 2 times higher. All but one of our pneumococcal isolates were resistant to three of more antimicrobial agents. There was incomplete concordance between high-level extended spectrum cephalosporin resistance and high-level penicillin resistance in our study. There were three pneumococcal isolates with high-level cephalosporin resistance and low-level penicillin resistance; and one isolate that was fully susceptible to penicillin (MIC = 0.01 μg/ml) but exhibited intermediate cephalosporin resistance. This latter *S pneumoniae* strain, serotype 19, was isolated from a 7-month-old male with primary bacteremia, and displayed penicillin resistance on disc diffusion testing . Other organisms with similar MIC profiles have been reported previously: a serotype 23 isolate from the CSF of a pediatric patient from California and another case report from Spain [19,20]. It has been suggested that strains like these may represent a unique clone of resistant pneumococcus differing in penicillin binding protein (PBP) patterns, serotypes, DNA sequences of the PBP genes, and/or cell wall compositions [21]. Unfortunately, our strain which was isolated in 1996 was not available for further molecular studies.

Due to the close correlation between resistance patterns, isolates of pneumococci demonstrating any degree of resistance to penicillin should be tested against clinically-important cephalosporins [2,3,17,18]. There is presently no acceptable disk diffusion test available for screening isolates for cephalosporin resistance. In our study, 80% of isolates were reported as being sensitive to both cefotaxime and ceftriaxone based on antibiotic disk susceptibility testing and yet all showed either intermediate or high-level resistance to these agents with MIC testing. Others have also reported this discrepancy between disk diffusion and MIC testing for cephalosporin-resistant pneumococcal strains [22]. Susceptibility screening using ceftizoxime

and cefuroxime disks does not identify strains with third-generation cephalosporins MICs of <4 and <2 μg/ml, respectively [22,23]. The 1 μg oxacillin disk screening test, which is both simple and cost-effective, is regarded as being highly sensitive but non-specific for the detection of penicillin resistance. The absence of a zone of inhibition around the disk is highly predictive of both penicillin and cephalosporin non-susceptibility. It has, therefore, recently been suggested that strains with growth up to the margin of the disk be reported as non-susceptible to both penicillin and extended spectrum cephalosporins, pending MIC testing [24].

The currently used broth microdilution method for determining MICs is accurate in predicting susceptibility but is time-consuming. The E-test agar diffusion MIC method yields results that are comparable to broth microdilution testing, but is easier to perform and read [25-28]. It may be a rapid and reliable alternative to conventional MIC testing of penicillin-resistant isolates, especially those from CSF.

In contrast to some gram-positive bacteria in which plasmid-mediated β-lactamases confer penicillin-resistance, these enzymes have not been detected in *S. pneumoniae* isolates [29]. Penicillin resistance in pneumococci is due to alterations in penicillin-binding proteins (PBPs) that are involved in the synthesis and modification of bacterial cell walls. These changes result in the formation of PBPs that have greatly reduced affinity for the antibiotic [30]. Genetic analysis has revealed that intermediate or high level penicillin resistance requires reductions in the affinities of PBP 1a, 2x, and 2b, whereas resistance to ceftriaxone and cefotaxime is due to altered forms of PBP 1a and 2x only [31,32]. Hence, resistance to extended-spectrum cephalosporins will not necessarily correlate with resistance to penicillin. Horizontal gene transfer of the *pbp* 2x and *pbp* 1a genes from a resistant to a susceptible strain was easily accomplished, the resulting transformant having high level extended spectrum cephalosporin

resistance [31,33]. Thus it is possible for rapid spread of resistant strains to occur within a pneumococcal population.

Nasopharyngeal carriage of pneumococci has been highly associated with age, and young age has been sited as a risk factor for the development of invasive penicillin-resistant pneumococcal infection in several studies [34,35]. Pneumococcal serotypes 6, 13, 19 and 23, which cause two-thirds of invasive pneumococcal disease in children, commonly infect the nasopharynx of young children and have been the most common serotypes isolated from children less than four years of age [36,37]. These serotypes are associated with prolonged (approximately 4 months), and intermittent carriage and rapid reacquisition. Approximately one in six children colonized by recently-acquired strains of pneumococci will develop invasive disease due to the strain [37]. Studies in Johannesburg show that the rate of nasopharyngeal carriage of antibiotic-resistant pneumococci is high in pediatric wards where nosocomially-acquired infections due to resistant strains often occur [38]. A survey of South African children below five years of age showed a high rate of nasopharyngeal carriage of intermediately-resistant *S pneumoniae* in 14.2% and 19.2% of children from urban and rural areas, respectively [14]. In this study, ceftriaxone and cefotaxime had MICs similar to that of penicillin G. These same authors found that during the period 1989-1990, 147 (41%) of 362 isolates from children < 4 years of age were found to be penicillin-resistant compared with 31 (6%) of 521 isolates from children > 4 years of age ($p < 0.000001$) [15]. Demonstrating the age-relatedness of resistant strains and invasive disease, in a study from South Africa the mean age of patients with pneumococcal bacteremia caused by penicillin-resistant strains was significantly lower (13.1 months) than that for patients with bacteremia caused by susceptible strains (31.6 months)[39]. This finding has been confirmed by our study – 62.5% were pediatric patients, the majority of whom were below 2 years of age.

In the present study forty percent of children and 47% of adults were HIV-seropositive, and HIV infection was the most important independent risk factor for antibiotic resistance in these age groups. A significant association between penicillin resistance and HIV-seropositivity has been previously documented for both adult and pediatric patients [40], and resistance to multiple antibiotics including third-generation cephalosporins seems to occur move frequently in HIV-seropositive children [40]. In addition, HIV infection has been found to be a significant risk factor for the development of invasive pneumococcal disease [41,42].

A study done to determine the impact of HIV infection and *S pneumoniae* bacteremia in a South African population revealed that HIV- seropositive children with bacteremia were significantly younger than HIV-seronegative children (mean age, 10 months vs 3.3 years, respectively;) [41]. This same finding was also true for adult subjects (mean age, 33.3 vs 46.6 years) in whom resistance to penicillin was also significantly increased. In our study patients, all of whom had invasive pneumococcal disease caused by antibiotic-resistant strains, those with HIV infection were younger than HIV-seronegative subjects. Malnutrition and failure to thrive may have been additional risk factors for the development of antibiotic-resistant pneumococcal disease in our HIV seropositive children. All HIV-seronegative children and adults had one or more other underlying illnesses that predisposed them to invasive pneumococcal infection. Other risk factors for antibiotic-resistant invasive pneumococcal disease in this group may have been frequent prior hospitalization, clinic attendance, and recent antibiotic therapy.

The majority of antibiotic-resistant pneumococcal infections in our study cohort were community-acquired (63%) rather than nosocomial (37%). This finding suggests that carriage of such organisms may be becoming more prevalent in the community [39], and may relate, in part,

to the increasing number of HIV-infected persons in the general population who are at higher risk for pneumococcal infections [3,40,41]. It should be noted, however, that one-third of patients with community-acquired infection had a history of recent hospitalization within the previous three months. It is possible that exposure to antibiotics and colonization with resistant pneumococci may have taken place during these prior hospital admissions. Twenty-nine percent of patients who acquired infection nosocomially had also been hospitalized within the three months preceding admission for their resistant pneumococcal infection; accordingly, the precise time and place of colonization of these individuals is uncertain. Nosocomial acquisition of multiply-resistant pneumococci has been well described [7,38]. Suppression of the normal microbial flora by the use of antibiotics, especially in an overcrowded hospital environment, may select for resistance and enhance colonization with resistant organisms.

Epidemiologic studies have shown that recent hospitalization and frequent therapeutic and prophylactic antibiotic use, especially in the prevention and treatment of otitis media, are risk factors for the acquisition and spread of drug-resistant pneumococcal strains [2,17-20,34,43]. One-third of our study patients had a history of recent antibiotic use and an additional 8% had probably also received antimicrobial therapy during previous hospitalization. Prolonged and relatively frequent hospitalization and concomitant antibiotic exposure may explain the higher incidence of infection with multiply-resistant strains in young children below 2 years of age [39]. Almost 90% of our study strains were resistant to trimethoprim-sulfamethoxazole on disk diffusion. Trimethoprim-sulfamethoxazole is offered as prophylaxis for *Pneumocystis carinii* infection to HIV-seropositive adults and children. In studies on children from more affluent suburbs in Johannesburg where trimethoprim-sulfamethoxazole is prescribed more frequently for a variety of pediatric infections, the rate of resistance to this drug among pneumococci was found to be higher than in the poor black township of Soweto [15].

Additionally, in HIV-infected adults the long-term use of trimethoprim-sulfamethoxazole has been hypothesized to promote the selection of penicillin-resistant strains of *S. pneumoniae* [44].

Numerous epidemiologic studies have also revealed a higher frequency of carriage and transmission of multiply-resistant pneumococci, including strains resistant to extended-spectrum cephalosporins, in institutional settings such as chronic care facilities, old age homes, and day care centers where close interpersonal contact and frequent antimicrobial drug usage co-exist [4,14,34,35,45-47]. Five of our study subjects (13%) had a history of hospitalization at a chronic care facility for tuberculosis. Three of these patients developed their resistant pneumococcal infection during their stay at this facility, and two subsequently died of severe nosocomial pneumococcal pneumonia.

The commonest clinical presentation in our study was bacteremic pneumococcal pneumonia with multilobar involvement on chest x-ray. The majority of patients whose clinical isolate was recovered from pulmonary secretions had co-pathogens. Both multilobar and polymicrobial pneumonia have been noted to be independent risk factors for mortality [3]. The rate of primary bacteremia in our study was low when compared to data from developed countries [48,49]. This is probably due to the fact that febrile patients who present without an obvious focus of infection are not routinely admitted for diagnostic workup in our setting, and hence rarely have blood cultures taken.

Only a limited number of published case series have focused on the clinical implication of third-generation cephalosporin resistance in pneumococcal disease [3,9,50]. In a retrospective review of children with pneumococcal bacteremia, those with ceftriaxone-susceptible and non-susceptible isolates displayed no statistically significant differences with respect to initial presentation with the exception of heart rate [9]. However, those children with non-susceptible

organisms were more likely to be febrile at follow-up and were also more likely to have an underlying chronic illness. These authors concluded that reduced susceptibility to ceftriaxone may be of little clinical significance in non-meningeal pneumococcal infections, and that antibiotic resistance among strains is not associated with increased virulence. Klugman and colleagues found that serum bactericidal activity of ceftriaxone against resistant pneumococci was excellent, suggesting that cephalosporin-resistant pneumococcal bacteraemia may be adequately treated with ceftriaxone and similar agents [52] . High-level penicillin resistance has been found to be an independent predictor of mortality among patients with pneumococcal bacteremia [13]. All our patients without bacteremia, whose initial empiric therapy was deemed inappropriate on the basis of *in vitro* susceptibility testing responded despite no change in antibiotic treatment. However, 3 of 16 (19%) bacteremic patients receiving inappropriate antibiotic therapy died of their infection ($p = 0.24$). Despite this lack of statistical significance, for critically ill patients with bacteremia and underlying disease we suggest that it may be prudent to add vancomycin to a third-generation cephalosporin if their pneumococcal isolate shows intermediate- or high-level cephalosporin resistance.

In a study of pediatric pneumococcal meningitis, it was found that the clinical courses and outcomes of children with penicillin-resistant isolates that had cefotaxime/ceftriaxone MICs of 0.5 μg/ml to 2 μg/ml were comparable to those with cephalosporin- and penicillin-susceptible isolates [50]. It was suggested that children with meningitis due to isolates with cefotaxime/ceftriaxone MICs $\leq$ 1 μg/ml may be adequately treated with these antibiotics. It has been determined that in order for effective bactericidal action, the antibiotic concentration in the CSF should exceed by 8 to 10 fold the MIC. Limited experience with adults suggests that the administration of a higher dose of cefotaxime may be effective in some patients with pneumococcal meningitis intermediately-resistant to extended spectrum cephalosporins [53].

However, several cases of pneumococcal meningitis treatment failure with third-generation cephalosporins have been documented over the years, and indicate that extended-spectrum cephalosporins may be ineffective as sole therapy for drug-resistant pneumococcal meningitis [2,3,17-21]. Antibiotics used to achieve clinical and bacteriological cure in these studies included vancomycin monotherapy; vancomycin plus chloramphenicol; vancomycin and rifampin with or without the addition of chloramphenicol; and imipenem. In these patients, the cefotaxime/ceftriaxone MICs ranged from 2 μg/ml to 16 μg/ml and the typeable isolates were all serotype 23. Out of the three patients with both clinical and microbiological evidence of meningitis in our study, two received appropriate combination therapy and clinically recovered. The patient who was treated with ceftriaxone monotherapy died.

In patients with severe pneumococcal pneumonia caused by penicillin- and cephalosporin-resistant pneumococci Pallares and colleagues reported a mortality of 26%, not significantly different from patients with susceptible strains, and patients treated with cefotaxime or ceftriaxone responded comparably regardless of the susceptibility of their isolate [3]. The mortality rate in our study cohort with cephalosporin-resistant pneumococcal infections, including bacteremia and meningitis, was surprisingly lower (10.5%). When we attempted to determine the impact of inappropriate therapy on clinical outcome, particularly in patients with meningitis, we found that the numbers in each of our groups (i.e., meningeal vs non-meningeal infection) were too small to permit statistically significant comparisons. All of the patients in our study cohort recovered except those initially admitted to hospital in critical condition. In multivariate analysis, Pallares and co-workers found the following to be independent prognostic factors for mortality: age $\geq$ 70 years; serious underlying illness; heart failure; multilobar involvement on chest radiograph; leukopenia; nosocomially-acquired pneumonia; and

polymicrobial pneumonia [3]. All four of our patients who died had one or more of these poor prognostic factors.

Therapeutic options for penicillin- and cephalosporin-resistant pneumococcal meningitis include vancomycin or rifampin (as part of combination therapy with ceftriaxone) and imipenem [19,51,52,54]. Limitations of vancomycin therapy include a narrow therapeutic margin in meningitis, variable blood brain barrier penetration and ventricular CSF levels, and the need to monitor serum concentrations. The administration of vancomycin at 30 mg/kg/day, together with adjunctive dexamethasone, was associated with four clinical failures in adult pneumococcal meningitis [51]. Only one of these patients had an isolate which was resistant to extended-spectrum cephalosporins (MIC 2 ug/ml). However, these patients received suboptimal doses of vancomycin. The recommended dose of vancomycin for penicillin-resistant pneumococcal meningitis is 60 mg/kg/day. It has been suggested that at this dose, vancomycin penetrates reliably into the CSF of children with acute meningitis and the range of CSF drug concentrations achieved is predictable [52]. The addition of vancomycin or rifampin to ceftriaxone resulted in a significantly enhanced CSF bactericidal activity against intermediate- or fully-resistant cephalosporin strains compared to ceftriaxone alone. However, in the same study, the serum bactericidal activity of ceftriaxone against resistant pneumococci was excellent, suggesting that cephalosporin-resistant pneumococcal bacteremia may be adequately treated using ceftriaxone and similar agents.

In a rabbit model of experimental meningitis caused by *S. pneumoniae* with a ceftriaxone MIC of 4 μg/ml, ceftriaxone combined with either vancomycin or rifampin resulted in enhanced CSF bactericidal activity [54]. Similarly, synergy with vancomycin plus ceftriaxone has been illustrated in other rabbit models of meningitis using two pneumococcal isolates, one partially-resistant and the other fully-resistant to ceftriaxone [55]. Against the more resistant strain the combination of ceftriaxone plus rifampin, with or without dexamethasone, resulted in prompt bacteriologic cure. It was suggested that in areas with a high prevalence of drug-resistant strains, initial empiric therapy for pneumococcal meningitis should include two antibiotics, ceftriaxone plus either vancomycin or rifampin. When adjunctive dexamethasone is used, the combination of ceftriaxone and rifampin is preferred, due to a significant reduction in the penetration of vancomycin into the CSF with dexamethasone co-administration [55]. Another study of experimental meningitis suggested that this effect of corticosteroids on CSF penetration may be circumvented by use of larger daily doses of vancomycin (i.e., 40 mg/kg/d rather than 20 mg/kg/d) [56].

In our study, 39% of isolates exhibited resistance to rifampin on disk diffusion screening. All six patients who had previously received or were currently on antituberculous therapy were infected with rifampin-resistant strains. Therefore, in such patients, rifampin should probably not be included in the initial empiric regimen for pneumococcal meningitis.

Imipenem plus the dihydropeptidase inhibitor, cilastatin at an iv dose of 100 mg/kg every six hours may be a useful alternative in the treatment of meningitis caused by multiply-resistant pneumococci [19]. However, use of imipenem-cilastatin is limited by its propensity to cause seizures (induced by the cilastatin component), especially in children with meningitis [57]. The carbapenem, meropenem may be a more suitable alternative as it does not require the addition of cilastatin.

The newer fluoroquinolones may play an important role in the future treatment of resistant pneumococcal infections. Gatifloxacin therapy was as effective as vancomycin plus ceftriaxone against a highly cephalosporin-resistant pneumococcal strain in an experimental rabbit meningitis model [58]. However, emergence of fluoroquinolone resistance among multiply-resistant pneumococcal strains has been described in Hong Kong [ ] and Canada [60], probably a result of selective pressure from increased fluoroquinolone use in these regions.

The use of chloramphenicol in South African children with penicillin-resistant pneumococcal meningitis was associated with increased morbidity and mortality due to its failure to achieve adequate CSF bactericidal activity [61]. Time-kill studies have shown antagonism between ceftriaxone and chloramphenicol in pneumococcal strains intermediately-resistant to ceftriaxone, and chloramphenicol should probably not be given in combination with a β-lactam antibiotic for these infections [62].

The predominant pneumococcal serotypes in our study was 23F, followed by serogroups 19 (mainly 19F) and 14 (mainly 14B). From 1987 to 1990, a dramatic increase in the prevalence of serogroup 23 *pneumococci resistant to penicillin* was observed in South Africa, compared to the surveillance period 1979-1986 [15]. During these periods, the commonest serotypes were 6A and 6B, followed by 19A and 14; serotype 19F was found to be of low prevalence among isolates [15]. Two penicillin-resistant pneumococcal clones of serotypes 19A and 6B, and a third multi-resistant serotype 19A clone were identified in 1997 [63]. These were described as being predominant in South Africa's penicillin-resistant pneumococcal population.

Serotype 23F, which originated in Spain, has subsequently been isolated in the United States, France, Turkey, and Mexico and intercontinental spread of a multiresistant clone has been described [64-70]. Molecular techniques have demonstrated that a multiresistant clone of *S pneumoniae* serotype 23F which is related to multiresistant isolates from Spain and South Africa has become disseminated in the U.S. [66]. There is also evidence suggesting *in vivo* capsular transformation of multiply resistant serotype 23F, where the resistant strain serves as the recipient of a DNA fragment, from a susceptible strain and encodes genes for synthesis of capsular polysaccharide [70].

Seventy percent of pediatric patients in our study were vaccine candidates due to underlying illness. The current 23-polyvalent pneumococcal vaccine, like other polysaccharide vaccines, is poorly immunogenic in children under the age of 2 years [71]. New pneumococcal conjugate vaccines which employ a number of carrier proteins

such as meningococcal outer membrane protein complex and a non-toxic diphtheria toxin mutant (CRM_{197}) appear capable of inducing adequate antibody levels in at-risk young children [72,73]. Forty percent of our adult patients were $\geq$ 60 years of age and could be classified as vaccine candidates based on age alone [46,47,74]. The protective efficacy of pneumococcal vaccine among the elderly may be limited, but it has shown to be efficacious and cost-effective in reducing the overall incidence of pneumococcal bacteraemia and morbidity in this age group [75,76].

None of the patients in our cohort had received pneumococcal vaccination prior to their study infection. Overall, 84% of patients were vaccine candidates based on age and/or the presence of underlying illness including 14 of I5 (93%) adults and 18 of 23 (78%) children. All our pneumococcal isolates were of serotypes that are included in the current 23-polyvalent vaccine and have been documented to cause invasive pneumococcal infection with resistant strains. This suggests that the present vaccine is appropriate for use in our region.

CHAPTER 5

CONCLUSIONS

Increasing efforts are needed to ensure accurate surveillance of penicillin and third-generation cephalosporin resistance among *S. pneumoniae*. Microbiology laboratories should routinely screen appropriate pneumococcal isolates for such resistance. Rapid and accurate methods for MIC determination such as the E-test are recommended for CSF isolates. Clinicians must have a heightened awareness of the prevalence of pneumococcal resistance to extended-spectrum cephalosporins. Modification of current empiric treatment guidelines for suspected pneumococcal meningitis may be necessary based upon local, regional, and national prevalences of cephalosporin resistance. Strategies must be developed to effectively increase coverage using the current 23-valent polysaccharide pneumococcal vaccine for at-risk persons $\geq$ 2 years old. The development of new polyvalent conjugate vaccines for children below 2 years of age is essential.

Table 1. Penicillin and third-generation cephalosporin resistance among blood and CSF isolates of *Streptococcus pneumoniae*, January 1995 to July 1997.

	Blood			CSF		
Year	Total isolates	No. of penicillin-resistant isolates (%)	No. of cephalosporin-resistant isolates (%)	Total isolates	No. of penicillin-resistant isolates (%)	No. of cephalosporin-resistant isolates (%)
1995	381	67 (18)	8 (2)	71	19 (27)	3 (4)
1996	415	120 (29)	6 (1.4)	60	14 (23)	0 (0)
1997	334	124 (37)	7 (2.1)	50	28 (56)	1 (2)
1998	518	161 (31)	3 (0.6)	84	24 (29)	1 (1.2)
1999	234	76 (32)	1 (0.4)	42	19 (45)	0 (0)
TOTAL	1,882	548 (29)	25 (1.3)	307	104 (34)	5 (1.6)

Table 2. Clinical and epidemiologic features in 40 patients with infections caused by third-generation cephalosporin-resistant *Streptococcus pneumoniae*.

Patient no.	Age/ sex	Underlying disease(s)	Culture site	Where acquired*	Previous hospitalization**	Prior antibiotics	Treatment	Outcome
1	3/F	Nephrotic syndrome	Blood	CA	No	---	AMPI + GENT	Recovered
2	1/F	HIV, malnutrition	Blood	CA	Yes	RIF	AMPI, CLOX, GENT, RIF	Recovered
3	46/F	HIV	Blood	N	Yes	RIF;CEFU; TMP-SMX	CEFU; ERY; TMP-SMX; AMIK; RIF	Died
4	2/F	Retinoblastoma, chemotherapy	Eye	N	No	ERY; CLOX	CEPH; topical tetra-cycline	Recovered
5	50/M	COPD	Bronc washings	CA	Yes	RIF	AMOX; then CEFU	Recovered
6	-/M	NK°	Blood, ascites	Nk°	NK°	NK°	NK°	NK°
7	1/M	-	CSF°°	N	Yes	NK°	ERY; CEPH	Recovered
8	1/F	HIV, malnutrition	Blood	CA	Yes	NK°	AMPI + GENT; then TMP-SMX	Recovered
9	3/M	Retinoblastoma, chemotherapy	Eye	N	No	AMOX; TMP SMX;ERY	CFT; RIF; then CIP; ERY; AMIK	Recovered

_contd.

10	-/M	NK°	Blood, CSF	NK°	NK°	NK°	NK°	NK°
11	28/F	Splenectomy, Castleman's disease	Blood, CSF	CA	No	---	CTX	Died
12	1/F	Malnutrition, measles	Tracheal aspirate	N	No	---	AMOX + CLOX; then CFT; AMIK_	Recovered
13	4/M	Retinoblastoma, chemotherapy	Eye	N	No	Topical CHLOR	CLOX, then RIF	Recovered
14	1/M	HIV, malnutrition, cardiomyopathy	Blood	CA	Yes	AMPI; GENT TMP-SMX	AMOX, then ERY	Recovered
15	2/F	Acute flaccid paralysis	Tracheal aspirate	N	Yes	NK°	CFT + AMIK, then CFT + VANC	Recovered
16	1/F	--	Blood	CA	No	---	AMPI, then AMOX	Recovered
17	4/M	Lymphoma	Blood	N	No	---	PIP-TAZ + AMIKI, then VANC	Recovered
18	3 mos/F	HIV	Blood	CA	No	---	TMP-SMX + AMPI, + RIF, then CFT + VANCO	Recovered
19	2/F	Retinoblastoma, chemotherapy	Eye	N	No	---	AMOX; then FUSI; topical mupirocin	Recovered
20	2/F	Biliary atresia	Blood	N	Yes	CFT	CTX	Recovered
21	1/F	HIV	Blood	CA	No	---	AMPI, then CTX, then ERY	Recovered

__contd.

22	1/M	HIV, malnutrition	Blood	CA	Yes	NK°	CFT; AMPI; TMP-SMX; then VANC	Recovered
23	67/M	COPD	Sputum	CA	No	---	AMOX + METR	Recovered
24	2/M	HIV, malnutrition	Blood	CA	Yes	TMP-SMX; AMOX	CEFU; ERY; then TMP-SMX	Recovered
25	1/M	HIV, malnutrition	Blood	CA	Yes	AMPI; AUGM; CEPH; TMP-SMX	CTX; TMP-SMX, then VANC	Recovered
26	60/F	HIV	Blood	CA	No	---	ERY + CEFU, then AMOX	Recovered
27	33/M	---	Blood	CA	No	---	CIP	Recovered
28	5mos/M	HIV	Blood	CA	No	---	CEFU + TMP-SMX, then AMOX	Recovered
29	67/M	COPD	Tracheal aspirate	N	No	NK°	CEFU; AUGM; CIP; then CFP	Recovered
30	63/M	COPD, alcoholism	Sputum	CA	No	---	AMOX, then ERY	Recovered
31	22/F	HIV	Blood	N	No	RIF	CIP + METR + TMP-SMX + RIF, then CEFU, then CFT	Died
32	32/F	HIV	Sputum	N	No	RIF	CEFU; RIF	Recovered

contd.

33	38/M	HIV	Blood, CSF	CA	No	---	CFT + RIF	Recovered
34	11/F	---	Blood, CSF	CA	No	---	CTX + VANC	Recovered
35	22/F	HIV	Blood	CA	No	---	CEFU	Recovered
36	3/M	----	Tracheal aspirate	N	No	---	CEFU; then PIP-TAZ + VANC	Recovered
37	3/M	HIV	Blood	CA	No	---	AUGM	Recovered
38	28/M	HIV	Sputum	CA	Yes	CEFU; RIF	TMP-SMX; ERY; RIF	Recovered
39	71/M	COPD, alcoholism	Sputum	CA	No	---	None	Recovered
40	76/F	Multiple myeloma	Blood	CA	No	---	CEFU + CLOX	Died

* CA = community-acquired; N = nosocomial.

** Within three months prior to isolation of the study organism.

+ AMIK = amikacin; AMPI = ampicillin; AMOX = amoxicillin; AUGM = amoxicillin-clavulanate; CEFU = cefuroxime; CEPH = cephalexin; CFP = cefipime; CFT = ceftriaxone, CHLOR = chloramphenicol; CIP = ciprofloxacin; CLOX cloxacillin; CTX = cefotaxime; ERY = erythromycin; FUSI = fusidic acid; GENT = gentamicin; METR = metronidazole; PIP-TAZ = piperacillin-tazobactam; TMP-SMX =trimethoprim-sulfamethoxazole; VANC = vancomycin.

o Not known.

oo Cerebrospinal fluid.

Table 3 Antibiotic susceptibility by disk diffusion in 40 patients with infections caused by third-generation cephalosporin-resistant *Streptococcus pneumoniae.*

	Susceptibility (% isolates)			
Antibiotic	Susceptible	Intermediate	Resistant	Not known (no. isolates)
Penicillin G	2.6	-	97.4	-
Ceftriaxone	80	6.7	13.3	9
Cefotaxime	80	6.7	13.3	9
Cefuroxime	80.8	7.7	11.5	13
Erythromycin	82.1	0	17.9	-
Clindamycin	82.1	0	17.9	-
TMP-SMX*	9.7	3.2	87.1	8
Tetracycline	26.3	23.7	50	1
Chloramphenicol	8	32	60	14
Rifampin	69.2	0	30.8	-
Ofloxacin	94.7	0	5.3	20
Vancomycin	100	0	0	7

* Trimethoprim-sulfamethoxazole.

Table 4 Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) for 40 strains of third-generation cephalosporin-resistant *Streptococcus pneumoniae.*

Antibiotic	MIC (μg/ml) Mean	MIC (μg/ml) Range	MIC breakpoint* I	MIC breakpoint* R	MBC (μg/ml) Mean	MBC (μg/ml) Range
Penicillin G	2.2	0.5-4	0.12-1	≥ 2	3.5	0.5-8
Cefotaxime	1.3	1-4	1	≥ 2	2.2	1-8
Ceftriaxone	1.3	1-4	1	≥ 2	1.8	1-8

*I = intermediate resistance; R = high-level resistance.

Table 5. Penicillin-resistant pneumococcal serotype distribution among 40 strains of cephalosporin- resistant *Streptococcus pneumoniae.*

Serotype	No. of cephalosporin-resistant isolates (%)
6	1 (2.5)
14	4 (10)
18	1 (2.5)
19	12 (30)
23	22 (55)
Total	40 (100)

Table 6. Underlying diseases in patients with invasive cephalosporin-resistant *Streptococcus pneumoniae* infection.*

Children (n = 23)	No. of cases (%)
HIV/AIDS	10 (44)
Malnutrition	7 (30)
Malignancy	
Retinoblastoma	4 (17)
Non-Hodgkin's lymphoma	1 (4)
Measles	1 (4)
Biliary atresia	1 (4)
Nephrotic syndrome	1 (4)
Dilated cardiomyopathy	1 (4)
Acute flaccid paralysis	1 (4)
Adults (n = 15)	
HIV/AIDS	7 (47)
COPD**	5 (33)
Alcoholism	2 (13)
Multiple myeloma	1 (7)
Splenectomy with Castleman's disease	1 (7)

* Some patients had more than one underlying disease.

** Chronic obstructive pulmonary disease.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Kularatne

CLEARANCE CERTIFICATE PROTOCOL NUMBER M6

PROJECT Invasive Disease Caused By Streptococcus Pnneumoniae Resistant To Penicillin & The Third Generation Cephalosporins

INVESTIGATORS Dr RS Kularatne

DEPARTMENT Clinical Infectious Disease..., Wits Medical School

DATE CONSIDERED 00/28/01

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 00/01/31 CHAIRMAN ..... (Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof R Smeço
Dept of Clinical Infectious Diseases, Wits Medical School

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE 31/01/00 SIGNATURE .....

PROTOCOL NO.: M 6

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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