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ANTIMICROBIAL SUSCEPTIBILITY IN COMMUNITY-ACQUIRED BACTERIAL PNEUMONIA IN ADULTS

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S. KARIUKI, J. MUYODI, B. MIRZA, W. MWATU and J.J.D. DANIELS

ABSTRACT

Objectives: To determine the antimicrobial susceptibility patterns of *Streptococcus pneumoniae* and *Haemophilus influenzae*, two bacterial pathogens commonly associated with community-acquired pneumonia.

Design: Cross-sectional study.

Setting: Bacterial isolates were obtained from adults suspected to have community-acquired pneumonia and who sought treatment at two city council clinics in Nairobi, Kenya. Susceptibility to antimicrobial agents was performed using a microdilution broth method, according to the criteria set by the National Committee for Clinical Laboratory Standards.

Results: A total of 277 *S. pneumoniae* and 58 *H. influenzae* were obtained from 536 adults examined in the period January 1998 to December 1999. Of the 277 *S. pneumoniae*, only 56.7% were susceptible to penicillin and 7.6% of strains were resistant to two or more antimicrobial agents. Of the 58 *H. influenzae* strains, 91.4% were sensitive to ampicillin, with 6.8% resistant to two or more antimicrobial agents. 8.6% were β -lactamase producers and accounted for the entire ampicillin-resistant population.

Conclusion: The prevalence of resistance to penicillin and other commonly used antibiotics among pneumococci is high and the large number of multi-resistant strains among *H. influenzae* is a cause for concern. The prudent use of antibiotics in treatment of pneumonia and other infections should be advocated to minimise spread of resistance.

INTRODUCTION

Respiratory tract infections are the most frequent reason for antibiotic prescription in ambulant patients. In most countries, *Streptococcus pneumoniae* and *Haemophilus influenzae* are the major causes of community-acquired pneumonia. Additionally in immunosuppressed patients, *Pseudomonas spp*, *Staphylococcus aureus* and *Pneumocystis carinii* may also be important causes of pneumonia. Indeed in sub-Saharan Africa, respiratory tract infections are the most common causes of morbidity and mortality in HIV-1 infected adults (1, 2). A recent study in hospitalised adults in Kenya with pneumonia (3) found that *S. pneumoniae* was documented in more than half of the patients while *H. influenzae* was the causative agent in 4% of patients.

Data from surveillance studies in the USA and Canada show that 15-57% of *S. Pneumoniae* isolates are intermediately or highly resistant to penicillin (4). Resistance to macrolide agents often occurs concurrently with penicillin resistance (5), and these too have been on the rise. Currently in several parts of the world including

the UK (6), Indonesia (7), South Africa (8), Brazil (9) and in four North African countries (10), the prevalence of multidrug resistant (MDR) pneumococci (resistant to 2 or more commonly used drugs including penicillin, doxycycline and cotrimoxazole) has been on the increase. However, these strains have remained fairly susceptible to newer quinolones and extended spectrum cephalosporins. In a recent study in Kenya (3) *S. pneumoniae* isolates from adults were either susceptible (MIC < 1 μ g/mL) or intermediate (1-2 μ g /mL) to penicillin. Resistance to erythromycin was 1.5%, to cotrimoxazole 54%, to doxycycline 32% and chloramphenicol 3%.

Resistance to β -lactams in *Haemophilus influenzae* is almost exclusively due to production of TEM-1 β -lactamases (95%) and ROB-1 enzymes (5%), both enzymes are plasmid-mediated. In 66.7% of recent *H. influenzae* isolates from USA and Canada the presence of ROB-1 correlated with cefaclor resistance that may be the result of mutations or change in expression in the ROB-1 β -lactamase gene (11). In the USA since 1983, the prevalence of β -lactamase producing *H. influenzae* has continued to rise from 14.5% to 37.5% in 1997, providing resistance to

penicillin and first generation cephalosporins (5). However, strains remained susceptible to extended spectrum cephalosporins.

Isolation, identification and susceptibility testing to guide treatment is not feasible for each individual patient because of the time required for the laboratory procedures. Treatment is therefore usually empirical. Empirical treatment should be based on the knowledge of local susceptibility levels of the most likely pathogens. These patterns must, therefore, be continually monitored, as resistance among both community and hospital acquired pathogens is increasing. The present study presents surveillance data on the antibacterial susceptibility of the key respiratory pathogens *S. pneumoniae* and *H. influenzae*.

MATERIALS AND METHODS

Patients: These were adults who sought care at the outpatient clinics at Mbagathi District Hospital and the Nairobi City Council Chest Clinic, and were treated for acute respiratory infections. Patients were recruited for a period of two years, January 1998 to December 1999. Specimens including sputum, and throat swabs were obtained from each case before treatment was commenced. In addition *S. pneumoniae* isolates were obtained from bronchoalveolar lavage and blood cultures from a total of 14 patients from two previous studies done at Kenyatta National Hospital and Mbagathi District Hospital (12, 13). For the bronchoscopy procedure patients were admitted to the Nairobi Hospital under the respective projects.

Laboratory procedures: Sputa were liquefied with dithiothreitol and diluted at a 1:1000 ratio with sterile distilled water, after which this dilution was inoculated onto blood and chocolate (Oxoid) plates. Similarly, throat swabs were inoculated onto blood and chocolate agar plates. 5 ml venous blood or bronchoalveolar lavage for culture was obtained from patients before antibiotic treatment was commenced and cultured in 50ml broth media containing brain heart infusion and para-aminobenzoic acid. Broth cultures showing signs of bacterial growth were subcultured onto blood, chocolate and macConkey agar plates. Inoculated plates were incubated at 37°C in a 5% CO₂ atmosphere for 24 hours. On blood agar *S. pneumoniae* were differentiated from other alpha haemolytic streptococci by including an optochin (ethylhydroxycupreine) disk; *S. pneumoniae* is inhibited by optochin. *S. pneumoniae* isolates were also confirmed by the bile solubility test. X and V factors required for growth of *H. influenzae* were used to confirm colonies of this bacterium subcultured from chocolate agar plates.

Antimicrobial susceptibility testing: Microdilution minimum inhibitory concentrations (MICs) were determined according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (14). The following antibiotics were used; penicillin (*S. pneumoniae* only), ampicillin (*H. influenzae* only), amoxicillin, amoxicillin/clavulanate (2:1), cefaclor, cefuroxime, cefixime, ceftriaxone, cefprozil, erythromycin, clarithromycin, azithromycin, ciprofloxacin, ofloxacin, chloramphenicol, doxycycline and cotrimoxazole. Two sets of 96-well microdilution trays were used; one for *S. pneumoniae* and the other for *H. influenzae*. Quality control organisms were tested for each microdilution run, and results of testing accepted only if quality control results were within limits specified by the NCCLS (14). Inoculum checks were always performed on all quality control organisms. The following quality control organisms were tested; *H. influenzae* (ATCC 49247), *H. influenzae* (ATCC 49766) and *S.*

pneumoniae (ATCC 49619). β -lactamase testing of all *H. influenzae* isolates was performed by the nitrocefin disk method (Difco). A positive control (a β -lactamase positive *H. influenzae*) and a negative control (such as *H. influenzae*, ATCC 10211) were used on each day of testing.

RESULTS

Bacterial isolates: During the two year study a total of 277 *S. pneumoniae* and 58 *H. influenzae* were obtained from 536 adults examined for suspected community-acquired pneumonia. Of these five *S. pneumoniae* and 2 *H. influenzae* were isolated from blood and BAL of separate patients (from a total of 14 from whom blood and BAL had been obtained). Out of a total of 15 patients from whom sputum could not be obtained in the course of the study three *S. pneumoniae* strains were isolated from throat swabs. In addition 16 patients carried both *S. pneumoniae* and *H. influenzae*, which were isolated from sputa.

Table 1

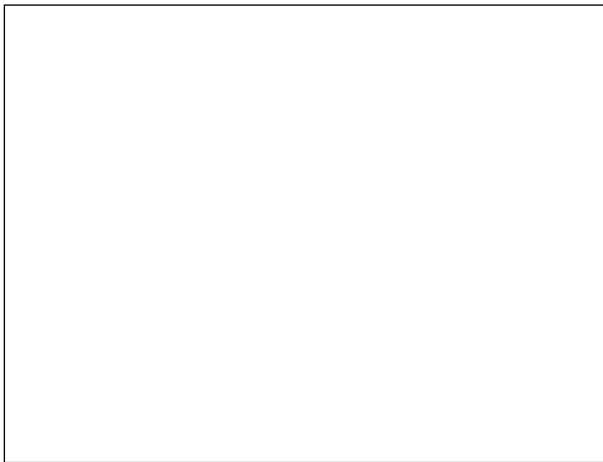
MICs (μ g/mL) of S. pneumoniae from community acquired pneumonia in adults, 1998-99 (n=277)

Antimicrobial Agent	Range	Mode	MIC ₅₀	MIC ₉₀
Penicillin	0.015-4	0.015	0.03	0.5
Amoxycillin	0.015-4	0.03	0.03	0.12
Amoxycillin/clavulanate	0.015-2	0.03	0.03	0.12
Cefaclor	0.5-64	0.5	1	2
Cefuroxime	0.015-8	0.03	0.12	1
Cefixime	0.12-16	0.25	0.5	4
Ceftriaxone	0.015-1	0.015	0.03	0.25
Cefprozil	0.12-16	0.12	0.25	0.5
Erythromycin	0.015-32	0.06	0.06	0.06
Clarithromycin	0.015-32	0.03	0.03	0.03
Azithromycin	0.03-32	0.06	0.06	0.12
Ciprofloxacin	0.5-4	1	1	2
Ofloxacin	1-8	2	2	2
Chloramphenicol	1-16	2	2	4
Doxycycline	0.06-8	0.12	0.12	8
Co-trimoxazole	0.06-8	4	1	8

Antimicrobial susceptibility of S. pneumoniae: Of the 277 *S. pneumoniae* strains studied there was good susceptibility to the β -lactam class of antimicrobials tested, except penicillin (43.3% non-susceptible), Cefaclor (27.8% non-susceptible) and cefixime (100% non-susceptible). The macrolides also fared well. The quinolones are more difficult to interpret, as there is no NCCLS breakpoint defined for ciprofloxacin. However, ofloxacin yielded 2.9% non susceptible strains. If a PK/PD-derived breakpoint is used for ciprofloxacin ($\leq 0.5 \mu$ g/mL) then 88.1% strains are non-susceptible. The older agents, namely chloramphenicol, doxycycline and co-trimoxazole, were not so effective with non-susceptibility rates of 3.2%, 24.2% and 50.5%, respectively. Figure 1 shows the percentage susceptibility of isolates and Table 1 is a summary of MIC values for *S. pneumoniae* strains.

Figure 1

Susceptibility patterns of S. pneumoniae isolates



What was particularly worrying was the high number of multidrug resistant strains (Table 3). Only 58.8% strains showed no resistance with 7.6% strains being resistant to two or more agents. The least effective antimicrobial agents were cefixime, ciprofloxacin (with PK/PD breakpoint of $\leq 0.5\mu\text{g/mL}$), co-trimoxazole, penicillin, cefaclor and doxycycline, with non-susceptibility rates of 100%, 88.1%, 50.5%, 43.3%, 27.8% and 24.2%, respectively. The most effective agent was amoxicillin (including amoxicillin/clavulanate) with 100% efficacy.

Table 2

MICs of H. influenzae from community acquired pneumonia in adults, 1998-1999 (n=58)

Antimicrobial agent	Range	Mode	MIC ₅₀	MIC ₉₀
Ampicillin	0.12-16	0.12	0.12	0.5
Amoxicillin	0.0125-16	0.5	0.5	1
Amoxicillin/clavulanate	0.12-1	0.25	0.5	0.5
Cefaclor	0.5-8	2	2	4
Cefuroxime	0.25-1	0.5	0.5	1
Cefixime	0.008-0.12	0.03	0.03	0.06
Ceftriaxone	0.004-0.008	0.004	0.004	0.008
Cefprozil	0.5-8	1	2	2
Erythromycin	1-8	4	4	8
Clarithromycin	2-16	8	8	8
Azithromycin	0.12-2	0.5	0.5	2
Ciprofloxacin	0.008-0.03	0.015	0.015	0.015
Ofloxacin	0.015-0.25	0.03	0.03	0.03
Chloramphenicol	0.25-8	0.5	0.5	0.5
Doxycycline	0.25-2	0.5	0.5	1
Co-trimoxazole	0.03-4	4	4	4

Antimicrobial susceptibility of H. influenzae: Of the 58 *H. influenzae* strains examined, only 8.6% produced β -lactamases, and these accounted for all the ampicillin-resistant strains (8.6%). The other β -lactams all showed excellent efficacy with > 90% susceptibility. The macrolides also demonstrated good efficacy

(clarithromycin and azithromycin > 90% susceptibility). However, no NCCLS breakpoint has been set for erythromycin, but if a PK/PD derived breakpoint of ≤ 0.25 is applied to the data 100% strains are resistant to this agent. Chloramphenicol and doxycycline show good efficacy of 94.8% and 100% susceptibility, respectively. Cotrimoxazole has only 44.8% susceptibility. Figure 2 shows the percentage susceptibility of isolates and Table 2 is a summary of MIC values for *H. influenzae*.

Figure 2

Susceptibility patterns for H. influenzae isolates



Multi-resistant strains were also prevalent amongst the haemophilus isolates (Table 3). All strains were non-susceptible to erythromycin (using a PKPD breakpoint of ≤ 0.25) and co-trimoxazole gave a resistance rate of 55.2%. All other antimicrobial agents tested gave non-susceptibility rates of <10%.

Table 3

Prevalence of multi-resistant strains 1998-99: percent of resistance to n antimicrobial agents

	<i>S. pneumoniae</i>	<i>H. influenzae</i>
Total strains	277	58
Resistant to 0 agents	58.8%	46.6%
Resistant to 1 agents	33.6%	46.6%
Resistant to 2 agents	6.5%	3.4%
Resistant to 3 agents	1.1%	3.4%
Resistant to 4 agents	0%	0%
Resistant to 5 agents	0%	0%
Resistant to 6 agents	0%	0%

DISCUSSION

In most countries, *Streptococcus pneumoniae* and *Haemophilus influenzae* are the major causes of community-acquired pneumonia (5, 9, 15). Furthermore, the prevalence of multidrug resistance (resistant to two or more antimicrobial agents) among *S. pneumoniae* has been on the increase worldwide (6-9) and our study observed a 7.6% prevalence of multidrug resistance. In addition, previous studies from Kenya found that

pneumococcal isolates did not show full resistance to penicillin although the prevalence of intermediate penicillin resistance has increased over the last two decades (3, 16). However, for treatment of acute pneumonia high dose penicillin (150,000- 200,000 units/kg/day) is still regarded as effective but the high number of non-susceptible isolates in this study may result in high clinical failure rates (3).

Only 1.8% of *S. pneumoniae* were resistant to penicillin while resistance to erythromycin and chloramphenicol was <5%, a much lower prevalence compared to figures from the industrialised countries (5). Of great concern from our study, however, was the high level of resistance to cotrimoxazole (38.3%) and doxycycline (14.8%). Similarly, Scott *et al* (3) observed prevalence of 54% and 32% to cotrimoxazole and tetracycline, respectively. These two drugs are among the most commonly available in most public hospitals for treatment of acute respiratory tract infections and other bacterial infections, and the high level of resistance would render them less effective for treatment of these infections. As in other studies from African countries (8, 10, 17) pneumococcal isolates from our study were susceptible to semi-synthetic penicillins and extended spectrum cephalosporins, but these drugs may be unavailable or are too expensive to be afforded by most patients who seek care at public hospitals in Kenya.

Resistance to β -lactams in *Haemophilus influenzae* is due to production of TEM-1 β -lactamases (95%) and ROB-1 enzymes (5%), both enzymes are plasmid-mediated. In the USA since 1983, the prevalence of β -lactamase producing *H. influenzae* rose from 14.5% to 37.5% in 1997, providing resistance to penicillin and first generation cephalosporins (10). In our study only 8.6% of *H. influenzae* produced β -lactamase although these were not characterised further. However, resistance to cotrimoxazole (51.7%), which is commonly used in outpatient treatment of acute respiratory tract infections, is worrying. Also of importance was the high prevalence of resistance to erythromycin (100%), which has been used in Kenya as second line treatment for acute respiratory tract infections. These trends may reflect overuse and sometimes misuse of these antimicrobials.

In conclusion our study observed that resistance to many of the first line antimicrobials used in Kenya (e.g. penicillin, cotrimoxazole, doxycycline, and erythromycin) for the treatment of community-acquired pneumonia is high, and that there is a trend to increasing resistance in the common aetiological agents. The World Health Organisation guidelines for treatment of outpatient acute respiratory tract infection recommend either cotrimoxazole or amoxicillin. Clearly for Kenya, cotrimoxazole would not be effective. However, this study does support the use of β -lactams. Furthermore, it may be worth considering a β -lactam/ β -lactamase inhibitor combination or a β -lactamase-stable cephalosporin due to the incidence of β -lactamase-producing *Haemophilus influenzae*. As it is generally well established that the emergence of resistance among bacteria is an inevitable

event following the use of antibiotics, the introduction of new and more potent antimicrobial agents will give an edge in this war but equally important will be efforts directed at delaying or minimising the likelihood for the emergence and subsequent dissemination of resistance by instituting prudent and appropriate use of available antimicrobials.

DECLARATION OF INTEREST

William Mwatu, a pharmacist and J.J. Daniels, a Clinical Microbiologist are employees of Glaxo-SmithKline. Both participated in the write-up of proposal for the study that was part of a larger multinational "Alexander Project" on surveillance of antibiotic resistance and in final report writing, hence qualify as authors. However, none of them was involved in the laboratory testing of antimicrobial susceptibility of isolates reported in this paper.

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