Antibiotic susceptibility of three major respiratory pathogens

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Abstract

The aim of the study is to determine the antibiotic susceptibility of *Moraxella catarrhalis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* to commonly used antibiotics and newer fluoroquinolones, extended-spectrum cephalosporins and macrolide.

A total of 33 *M. catarrhalis*, 201 *H. influenzae* and 148 *S. pneumoniae* strains isolated from clinical specimens were subjected to antibiotic susceptibility testing by determination of minimum inhibitory concentration (MIC). MIC of antibiotics was determined using agar dilution method as outlined by NCCLS.

S. pneumoniae resistant to penicillin was seen in 9.6% of isolates; susceptibility of *M. catarrhalis* to ampicillin and tetracycline was 21% and 97% respectively. The majority of the *H. influenzae* (91%) was still susceptible to chloramphenicol. Trovafloxacin was the most effective antibiotic against the 3 organisms tested, as all isolates tested were susceptible. Ciprofloxacin and cefepime were equally active against *M. catarrhalis*. Cefepime was more effective than fluoroquinolones against *S. pneumoniae*. Of the two fluoroquinolones tested, resistance to ciprofloxacin has emerged in Malaysian *S. pneumoniae* isolates.

Key words: Respiratory pathogens; *Moraxella catarrhalis*; *Haemophilus influenzae*; *Streptococcus pneumoniae*

Introduction

Respiratory tract infections (RTIs) are the commonest cause of illness of all ages globally. Important RTIs include, acute pharyngitis, acute otitis media, acute exacerbation of chronic bronchitis, acute/chronic sinusitis and milder form of pneumonia. Lower respiratory tract infections (LRTIs) are the most common diseases of humans with approximately 5 per thousand per year suffering from a persistent infection (Garibaldi, 1985). Community acquired pneumonia is a major problem, and is the sixth highest cause of death worldwide (Barlett *et al.*, 1995). Major bacterial pathogens isolated from respiratory tract infections include *S. pneumoniae*, *H. influenzae*, *Haemophilus* spp. and *Moraxella catarrhalis* (Marrie, 1994; Smith *et al.*, 1976).

The use of appropriate antimicrobial is vital because even mild respiratory tract infections can potentially lead to serious complications. Ten to 20 years ago it was much easier to treat respiratory tract infections because resistance to commonly used antibiotics was rare. However resistance has now increased to such an extent that many previously used antibiotics can no longer be effective.

The aim of the study is to determine the antibiotic susceptibility of *M. catarrhalis*, *S. pneumoniae* and *H. influenzae* to commonly used antibiotics and to newer fluoroquinolones, extended-spectrum cephalosporins and macrolide.

Materials and Methods

Bacterial strains

A toral of 33 *M. catarrhalis* were isolated in 1991-1993 from sputum of patients with pneumonia where no other pathogens were isolated. The strains were identified by their ability to utilise carbohydrates, presence of oxidase, catalase and deoxyribonuclease enzymes.

S. pneumoniae and *H. influenzae* were clinical isolates in 1995 to 1997 from blood or sputum of patients presenting with pneumonia. A small proportion of the *S. pneumoniae* and *H. influenzae* strains were isolated from cerebrospinal fluid (CSF) obtained from 5 state hospitals in the country. The colony morphology and gram stain appearance were used for strain identification; *S. pneumoniae* was identified by susceptibility to optochin, and *H. influenzae* by requirement for X & V factors.

All the strains were stored in glass beads coated with glycerol broth and kept at -70°C until tested.

Antibiotics

All the 3 organisms were tested against cefuroxime, cefepime, ciprofloxacin and trovafloxacin. *M. catarrhalis* and *H. influenzae* were also tested against ampicillin and tetracycline. Chloramphenicol was tested alone against *H. influenzae*, and azithromycin, penicillin G, cefaclor and ceftriaxone were tested against *S. pneumoniae* alone. The antibiotic powders were obtained

from Bristol-Myers Squibb (cefepime), Pfizer (trovafloxacin), Bayer (ciprofloxacin) and SIGMA (other antibiotics).

Minimum Inhibitory Concentration

Susceptibility of the isolates to antibiotics was determined by minimum inhibitory concentration (MIC) using agar dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

The test medium used was Mueller-Hinton (MH) agar for *M. catarrhalis*, Mueller-Hinton blood agar (MHBA) for *S. pneumoniae* and *Haemophilus* test medium (HTM) for *H. influenzae*. Direct bacterial suspensions with turbidity equal to 0.5 Mac Farland standard were prepared from an overnight culture. The suspensions were diluted 10 fold to serve as the inoculum and plates were inoculated using a Denley multipoint inoculator which delivered approximately 10^4 colony-forming units. The test was run simultaneously with control organism *S. aureus* ATCC 25913, *E. coli* ATCC 25922 and *Ps. aeruginosa* ATCC 27853. After inoculation the plates were incubated at 35°C for 18 hours before being read. *H. influenzae* and *S. pneumoniae* were incubated in 5-10% CO_2. The MIC was defined as the concentration of antibiotic that inhibited all visible growth on the plate.

Results

The results were expressed as the MIC range, MIC_{50} and MIC_{90}. The MIC_{50} and MIC_{90} are the concentrations of the antibiotic that inhibited 50% and 90% of the strains tested respectively. Only 21.2% of *M. catarrhalis* tested were susceptible to ampicillin and 96.8% were sensitive to tetracycline. All were susceptible to extended spectrum cephalosporins, cefuroxime and cefepime. MIC_{90} of cefepime was one dilution lower than cefuroxime, at 1.0 µl/ml. For flouroquinolones, susceptibility was 100% with MIC_{50} and MIC_{90} much lower for trovafloxacin compared to ciprofloxacin (Table 1). The MIC_{90} of trovafloxacin against *M. catarrhalis* was 0.0078 mg/ml i.e. 3 dilutions lower than ciprofloxacin, and 7 dilutions lower than cefepime.

Of the 201 strains of *H. influenzae* tested, only 76.6% were inhibited by ampicillin at concentration (1.0 µg/ml, the breakpoint for susceptibility to ampicillin. Susceptibility to both cefuroxime and cefepime was 99.5% but the MIC_{90} was 1.0 µg/ml and 0.125 µg/ml respec-

Table 1: Activity of selected antimicrobial agents against *M. catarrhalis*, *H. influenzae* and *S. pneumoniae*

Antimicrobial agents	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)	% susceptible
M. catarrhalis (33)				
ampicillin	0.5	4.0	<0.0313-4.0	21.2
cefuroxime	1.0	2.0	0.25–2.0	100
trovafloxacin	0.0039	0.0078	0.039-0.0156	100
tetracycline	0.5	1.0	0.0156-0.25-16.0	96.77
ciprofloxacin	0.03125	0.0625	0.0313-0.125	100
cefepime	0.25	1.0	0.0625-1.0	100
H. influenzae (201)				
ampicillin	0.25	8.0	0.0625->8.0	76.62
cefepime	0.0625	0.125	0.0156->6.0	99.51
chloramphenicol	0.5	1.0	0.125-16.0	91.55
ciprofloxacin	0.0078	0.0156	0.0039->0.5	99.51
tetracycline	0.5	2.0	0.125->16.0	89.05
cefuroxime	0.5	1.0	0.125-8.0	99.51
trovafloxacin	0.0039	0.0156	0.001->0.125	100
S. pneumoniae (148)				
azithomycin	0.047	0.125	0.016->32.0	95.95
amoxicillin-clavulanate	0.016	0.023	0.010-6.0	99.33
cefuroxime	0.023	0.064	0.016-8.0	97.31
ceftriaxone	0.023	0.064	0.004-1.5	98.65
penicillin G	0.016	0.047	0.002-4.0	90.56
cefaclor	0.50	1.0	0.25->32	91.89
cefepime	0.0156	0.03225	0.0078-2.0	99.71
ciprofloxacin	0.5	1.0	0.25-8.0	98.27
trovafloxacin	0.064	0.125	0.032-0.25	100

tively. More than 91% of *H. influenzae* tested were susceptible to chloramphenicol. The MIC_{90} of trovafloxacin and ciprofloxacin for *H. influenzae* was 0.0156 µg/ml but susceptibility to trovafloxacin and ciprofloxacin was 100% and 91.5% respectively.

Among the *S. pneumoniae* strains, penicillin susceptibility was 90.6% with MIC_{50} at 0.047 µl/ml. Susceptibility to cephalosporin was higher, between 91.9% for cefaclor and 99.7% for cefepime (Table 1). Addition of β-lactamase inhibitor improved the performance of penicillin group with 99.3% being susceptible to amoxicillin-clavulanate. Among the flouroquinolones, trovafloxacin demonstrated a better activity compared to ciprofloxacin where the MIC_{90} was at 0.125 µl/ml (Table 1). Cephalosporins were more active towards *S. pneumoniae* compared to flouroquinolones with MIC_{90} at 0.0313 µl/ml and 0.064 µl/ml for cefepime and cefuroxime respectively.

Discussion

Beta-lactamase-mediated amoxicillin resistance in *H. influenzae* and *M. catarrhalis* together with altered penicillin-binding-protein mediated penicillin resistance in *S. pneumoniae* have increased significantly in the past decade. β-lactamase-mediated amoxicillin resistance between 20-40% of *S. pneumoniae* isolates were reported in Europe and North America and almost 100% of *M. catarrhalis* were β-lactamase producets (Jorgensen, 1992; Kayser *et al*, 1990; Doern, 1995). The rate of ampicillin resistance amongst the *H. influenzae* strains tested in this study was much higher compared to the National Surveillance data which was 16.4% in 1995 (Rohani *et al*., 1999). The prevalence of penicillin-resistant pneumococci in Asia in the 1980s was 2% in Malaysia (Cheong *et al*., 1988) and 8-12% in Pakistan and Bangladesh (Appelbum, 1992). The rates have risen in the 1990s, and in this study the resistance rate was 9.6%.

The rate of ampicillin resistance among the isolates of *M. catarrhalis* tested was comparable to that of the Alexander Project which found more than 90% β-lactamase producer in both European and United States Centers (Gruneberg *et al*., 1996). We observed a 78.8% ampicillin resistance among the *M. catarrhalis* strains in this study.

High levels of resistance to fluoroquinolones in these 3 most common respiratory pathogens are emerging in many parts of the world. For example, MIC to ciprofloxacin of 32 µg/ml or greater is no longer a rare occurrence in the Western region of the United States and Europe for *H. influenza* and *M. catarrhalis* (Gould *et al*., 1994; Barriere *et al*., 1993; Corkill *et al*., 1994; Cunliffe *et al*., 1995) and most currently available fluoroquinolone have marginal potency against streptococci such as pneumococci. In this study ciprofloxacin MIC of >32 µg/ml was observed in *S. pneumoniae*.

Trovafloxacin, 7-(3-asabicyclo [3,1,0] hexyl) - naphthyridano is a synthetic fluoroquinolone antibiotic which possesses a broad spectrum activity against gram positive and gram negative bacteria, including those resistant to ciprofloxacin (Eliopoulas *et al*., 1993; Gooding *et al*., 1993). This was observed among both *H. influenzae* and *S. pneumoniae* but for *H. influenzae* the two flouroquinolones were equally active.

Cefepime is a new semi-synthetic cephalosporine which has been shown to have high *in vitro* activity against Gram negative organisms and methicillin sensitive *S. aureus* (Bodey *et al*., 1985; Lim *et al*., 1993). Our finding showed that cefepime was equally active against *M. catarrhalis* and *H. influenzae*, as well as for *S. pneumoniae*.

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