SHORT ARTICLE

In-vitro effects of penicillin and clindamycin on the expression of *Streptococcus pneumoniae* capsule

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The effects of subinhibitory concentrations of penicillin or clindamycin were evaluated in 20 isolates of *Streptococcus pneumoniae* that were fully susceptible to penicillin and in 20 isolates that were of intermediate resistance. All isolates were capsulate and susceptible to clindamycin. After incubation in one-half of the MIC of clindamycin, 17.5% of isolates retained a capsule, compared to 87.5% after incubation with one-half of the MIC of penicillin. Clindamycin appears to be superior to penicillin in reducing the expression of the capsule by *S. pneumoniae*.

Introduction

Streptococcus pneumoniae is a common cause of acute otitis media, pneumonia and meningitis. Isolates that exhibit intermediate susceptibility (MIC 0.1–1.0 mg/L) or resistance (MIC ≥ 2 mg/L) to penicillin are increasingly prevalent and this complicates the management of infections caused by this organism [1]. Penicillin-resistant *S. pneumoniae* may be resistant to other commonly prescribed antibiotics such as macrolides, some cephalosporins, and co-trimoxazole [2, 3], but most remain susceptible to clindamycin [1, 4].

The virulence of *S. pneumoniae* is partly attributed to its capsule which enables it to escape phagocytosis [5]. Interruption of capsule production in *S. pneumoniae* by the use of transposon mutagenesis renders the organism avirulent [6].

The influence of subinhibitory concentrations of penicillin and clindamycin on the expression of capsule by *S. pneumoniae* *in vitro* were investigated.

Materials and methods

S. pneumoniae strains were isolated from throat cultures of children seen in a paediatric clinic. The organisms were identified by characteristic colony

morphology and α -haemolysis, confirmed by susceptibility to ethylhydrocupreine (Optochin[®]) and bile solubility; they were serotyped by a capsule swelling test with type-specific antiserum [7]. Routine screening of penicillin susceptibility was with a 1-mg oxacillin disk on sheep blood-supplemented Mueller-Hinton agar. A strain was suspected of penicillin resistance when the zone of inhibition surrounding the disk measured <20 mm after incubation for 24 h without CO₂ at 35°C. The MICs of penicillin (Penicillin-G, Bristol-Myers, Squibb, Princeton, NJ, USA) and clindamycin (The Upjohn Company, Kalamazoo, MI, USA) for each isolate were determined by the agar dilution method. Mueller-Hinton agar supplemented with sheep blood 5% was used, as recommended by the National Committee for Clinical Laboratory Standards [8]. The final inocula contained (1×10^4) – (3×10^4) cfu/spot. The MIC was defined as the lowest drug concentration that prevented visible growth or yielded fewer than six discrete colonies.

To investigate the effect of subinhibitory concentrations of penicillin or clindamycin on capsule formation, isolates of *S. pneumoniae* (10^6 cfu in 1 ml) were incubated for 48 h at 37°C in 9 ml of Todd-Hewitt broth medium that included antibiotic at 0.5 MIC. A tube without antimicrobial agents served as a control. The presence of a capsule was evaluated by electron microscopy after staining with ruthenium red [7]. The specimens were coded to prevent observer bias. An isolate was considered to be capsulate if a capsule was seen in $>75\%$ of 500 bacterial cells.

Statistical analysis was done by the χ^2 test and Fisher's exact test.

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Table 1. Effects of subinhibitory concentrations of penicillin and clindamycin on production of capsule by 40 strains of *S. pneumoniae* after incubation for 48 h

Susceptibility to penicillin	Number of capsulate strains (%) after incubation with		
	Penicillin	Clindamycin	Saline
Fully susceptible (n = 20)	17 (85)	3 (15)*	20 (100)
Intermediate resistance (n = 20)	18 (90)	4 (20)*	20 (100)

*p < 0.05 compared with penicillin results.

Results

Forty isolates of *S. pneumoniae* were included in the study; 20 were fully susceptible to penicillin and 20 exhibited an intermediate level of resistance. All isolates were susceptible to clindamycin (MIC 0.025–0.05 mg/L).

Twelve isolates were serotype 6 (seven susceptible and five intermediately resistant to penicillin), eight were serotype 14 (three susceptible and five intermediate), 10 were serotype 19 (six susceptible and four intermediate), and 10 were serotype 23 (five susceptible and five intermediate).

All isolates were capsulate when grown without antibiotics. Incubation with clindamycin significantly reduced the prevalence of capsule formation, as compared with penicillin in both penicillin susceptible or intermediately resistant isolates (Table 1).

Discussion

In this study, exposure of *S. pneumoniae* to clindamycin at 0.5 MIC commonly suppressed the formation of the capsule, whereas a subinhibitory concentration of penicillin was less effective in this respect. The inability of penicillin to suppress capsule formation has been demonstrated previously in experiments in which the concentration of *S. pneumoniae* in the growth medium was $>10^6$ – 10^7 cfu/ml [9].

Similar suppressive effects of subinhibitory concentrations of clindamycin on capsule formation have been observed in *S. pyogenes* [10] and *Staphylococcus aureus* [11]. In the latter study, phagocytosis of *Staph. aureus* by macrophages was enhanced [11].

The growing resistance of *S. pneumoniae* to penicillin and other antimicrobial agents necessitates the use of alternative effective drugs. Most intermediately resistant *S. pneumoniae* isolates are currently susceptible to clindamycin [1, 4] and suppression of capsule formation is an additional beneficial quality that might enhance eradication of the organism. Concentrations of clindamycin in the middle ear [12] are c. 100 times higher than the MIC₉₀ of penicillin-resistant *S.*

pneumoniae. Clindamycin is effective in the therapy of otitis media caused by penicillin-susceptible and penicillin-resistant *Staph. aureus*, *Staph. epidermidis* [13], *S. pneumoniae* and anaerobic bacteria [14]. Further studies are warranted to evaluate the clinical efficacy of clindamycin in other *S. pneumoniae* infections.

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