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Meropenem susceptibility of *Neisseria meningitidis* and *Streptococcus pneumoniae* from meningitis patients in The Netherlands

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In-vitro susceptibility of 299 *Neisseria meningitidis* and 157 *Streptococcus pneumoniae* strains from meningitis patients in The Netherlands in 1993 and 1994 to meropenem was determined using the Etest. Susceptibility to penicillin, ceftriaxone, and chloramphenicol was also determined. Rifampicin susceptibility was additionally tested for *N. meningitidis*. Of the meningococci, 4.3% were of intermediate resistance to penicillin and 0.3% were resistant to rifampicin. One pneumococcal isolate (0.6%) was of intermediate resistance to penicillin. All strains were susceptible to meropenem. We conclude that meropenem is *in vitro* highly active against *N. meningitidis* and *S. pneumoniae*.

Introduction

Bacterial meningitis in The Netherlands is caused predominantly by *Neisseria meningitidis* and *Streptococcus pneumoniae*. The incidence of *Haemophilus influenzae* type b meningitis has decreased strongly as a result of vaccination against this bacterium during the last 3.5 years.[1]

Although the prevalence of resistance of *N. meningitidis* and *S. pneumoniae* to penicillin, chloramphenicol, or cephalosporins in The Netherlands is low,[1] the resistance of these organisms to antibiotics commonly used in the treatment of meningitis is increasing globally.[2,3] The prevalence of penicillin resistant *N. meningitidis* is low in The Netherlands,[1] the UK (3%)[4] andthe USA (4%).[5] The prevalence of such strains in Spain, however, is 20–50%.[6] Pneumococcal resistance to penicillin due to changes in its penicillin binding proteins was first reported in 1965.[3] The prevalence of such resistance was limited until an epidemic of highly resistant pneumococci occurred in South Africa in 1977.[3] Since then, resistance has developed worldwide and in some regions it occurs in a frequency of up to 70%.[7] Reports of reduced susceptibility of pneumococci to several antibiotics, including broad-spectrum cephalosporins, have also recently been published.[7]

Therefore it is essential to investigate the bacterial susceptibility of meningitis isolates to other classes of antibiotics appropriate for the treatment of patients with meningitis. The carbapenem meropenem excels in in-vitro activity against a broad range of bacteria including penicillin resistant pneumococci,[8] is stable to the majority of β-lactamases, and penetrates well into the CSF.[9] Furthermore, meropenem seems to lack the potential of causing seizures as has been reported for imipenem-cilastatin, a structurally related carbapenem.[9] We tested the in-vitro susceptibility to meropenem of 299 *N. meningitidis* and 157 *S. pneumoniae* strains isolated from patients with meningitis.

Materials and methods

Bacterial strains and culturing conditions

N. meningitidis and *S. pneumoniae* isolates were collected by The Netherlands Reference Laboratory for Bacterial Meningitis of the Academic Medical Center (Amsterdam, The Netherlands) and of the National Institute of Public Health and the Environment (Bilthoven, The Netherlands), during 1993 and 1994. Approximately 75% of all bacterial meningitis isolates are sent by the clinical microbiology laboratories to the reference laboratory. In total 1136 meningococcal strains and 398 CSF pneumococcal strains were received in 1993 and 1994.[1] From this collection 456 strains, 299 *N. meningitidis* and 157 *S. pneumoniae*, were used to determine in-vitro susceptibility. All *S. pneumoniae* isolates were obtained from CSF. Of *N. meningitidis* isolates, 126 were obtained from patients with meningitis. The remaining 173 *N. meningitidis* isolates were isolated from blood cultures of patients having meningococcal septicaemia.

All isolates were identified upon receipt using standard

procedures, were stored at −70°C in glycerol-based medium on glass beads. One or two beads were removed from stock cultures, subcultured on to chocolate agar plates (*N. meningitidis*) or blood agar plates (*S. pneu - moniae*), and incubated for 18–24 h at 35°C in air with 5% CO_2. The quality control (QC) strains used in this study were *Escherichia coli* ATCC 4922, *Staphylococcus aureus* ATCC 29213, *H. influenzae* ATCC 49247 and *S. pneu - moniae* ATCC 49619, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, Villanova, PA, USA).[10] QC testing was performed daily for 3 days and then weekly.

Susceptibility testing

For susceptibility testing the Etest was used.[10] The inoculation procedure was performed according to the NCCLS guidelines.[10] The inocula were prepared by suspending bacteria in phosphate-buffered saline to achieve a turbidity equivalent to a 0.5 McFarland standard. A sterile, cotton swab was dipped into the bacterial suspension and the entire surface of an agar plate was swabbed four times, resulting in a confluent lawn of growth. For meningococci chocolate Mueller–Hinton agar (Oxoid Ltd, Basingstoke, UK) was used and for pneumococci Mueller–Hinton 5% sheep blood agar (Oxoid Ltd). All plates were incubated at 35°C in 95% air and 5% CO_2 for 18–24 h. The MIC value was read where bacterial growth intersected the Etest strip. The MIC criteria used for the Etest were those used for microorganisms tested by dilution susceptibility test methods (NCCLS).[10] Criteria for susceptibility to penicillin and rifampicin for meningococcal isolates have been suggested elsewhere,[2,4] and results for chloramphenicol and ceftriaxone were interpreted according to the strictest NCCLS criteria for other microorganisms.[10] For determination of the susceptibility category, the Etest results were rounded up to the nearest two-fold dilution value, as recommended by the manufacturer. The susceptibility of *N. meningitidis* to meropenem, penicillin, ceftriaxone, chloramphenicol, and rifampicin, and of *S. pneumoniae* to meropenem, penicillin, ceftriaxone, and chloramphenicol was tested.

Results

The penicillin, ceftriaxone, and chloramphenicol susceptibility data for *N. meningitidis* and *S. pneumoniae*, as well as the rifampicin susceptibility data for *N. meningitidis*, are shown in the Table. Thirteen *N. meningitidis* isolates (4.3%) were of intermediate resistance to penicillin (MIC 0.1–1 mg/L); all were susceptible to ceftriaxone (MIC $\leq$ 0.25 mg/L) and chloramphenicol (MIC $\leq$ 2 mg/L). One meningococcal strain (0.3%) was resistant to rifampicin (MIC $\geq$ 4 mg/L) and one *S. pneumoniae* strain (0.6%) was of intermediate resistance (MIC 0.1–1 mg/L) to penicillin. The 456 tested strains have been included in a previous report of the antimicrobial susceptibility of isolates.[1]

All strains of *N. meningitidis* and *S. pneumoniae* were susceptible to meropenem. The distribution of MICs of meropenem for *N. meningitidis* and *S. pneumoniae* is shown in the Figure. There was no difference in the MIC values of meropenem for *N. meningitidis* (MIC_{50} of 0.006 mg/L and MIC_{90} of 0.012 mg/L in 1993; MIC_{50} of 0.006 mg/L and of 0.008 mg/L in 1994) or *S. pneumoniae* (MIC_{50} of 0.012 mg/L, and MIC_{90} of 0.016 mg/L) between 1993 and 1994. Furthermore, no difference was found between MIC values for CSF and blood isolates of *N. meningitidis* (MIC_{50} of 0.006 mg/L and MIC_{90} of 0.008 mg/L for CSF isolates; MIC_{50} of 0.006 mg/L and MIC_{90} 0.012 mg/L for blood isolates). The MIC_{50} and MIC_{90} of meropenem for meningococcal strains intermediately resistant to penicillin were 0.012 mg/L and 0.023 mg/L, respectively. The MIC of meropenem for the meningococcal strain resistant to

Table. Antimicrobial susceptibility of 299 CSF and blood isolates of *N. meningitidis* and 157 CSF isolates of *S. pneumoniae* to penicillin, ceftriaxone, chloramphenicol, and rifampicin

	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)
N. meningitidis			
penicillin	0.032	0.064	0.006–0.190
ceftriaxone	0.000	0.000	0.000–0.003
chloramphenicol	0.750	1.00	0.038–2.00
rifampicin	0.012	0.023	0.000–0.012[a]
S. pneumoniae			
penicillin	0.016	0.023	0.006–0.250
ceftriaxone	0.016	0.023	0.003–0.094
chloramphenicol	2.00	3.00	0.250–4.00

[a] One meningococcal strain was resistant to rifampicin with MIC > 256 mg/L.
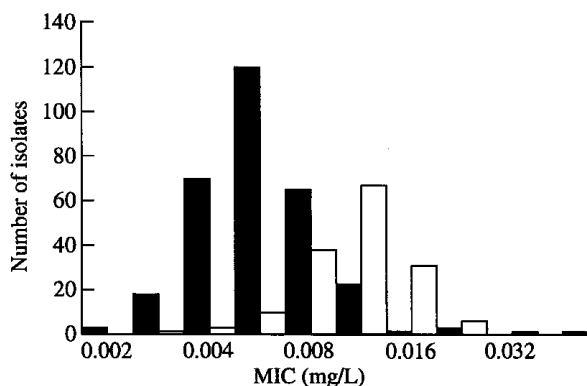
Figure. Distribution of MICs of meropenem for *N. meningitidis* and *S. pneumoniae* isolates. ■ *N. meningitidis* (*n* = 299); □ *S. pneumoniae* (*n* = 157).

rifampicin was 0.003 mg/L. The MIC of meropenem for the pneumococcal strain intermediately resistant to penicillin was 0.047 mg/L.

Discussion

Meropenem appears to be a rational addition to the therapeutic options for the treatment of patients with bacterial meningitis. It is, *in vitro*, highly effective against *N. meningitidis* and *S. pneumoniae* isolates causing meningitis and has, in contrast to third-generation cephalosporins, activity against some of the more unusual pathogens causing meningitis, particularly *Listeria monocytogenes*.[9]

Previously we reported the susceptibility of *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* isolates from meningitis cases to various antimicrobials.[1] The frequency of resistance to penicillin, ceftriaxone, chloramphenicol, and rifampicin amongthese isolates is very low. The strains tested for meropenem have been included in this previous survey.

All tested *N. meningitidis* and *S. pneumoniae* strains were susceptible to meropenem, including thirteen *N. meningitidis* strains of intermediate resistance topenicillin, the *N. meningitidis* strain resistant to rifampicin, and the *S. pneumoniae* strain of intermediate resistance to penicillin. As meropenem may be especially useful in the management of meningitis caused by meningococcal and pneumococcal strains resistant to these antibiotics, ideally a larger number of resistant strains should have been tested for susceptibility to meropenem. However, in a recent report the MICs of meropenem for three pneumococcal strains of intermediate resistance (MIC values of penicillin 0.125–1 mg/L) and twelve penicillin resistant strains (MIC values of penicillin $\geq$ 2 mg/L) were low, with MICs of meropenem ranging from 0.015–0.5 mg/L.[8] Interestingly, in our study

MICs of meropenem for penicillin resistant isolates were somewhat higher than MICs of penicillin susceptible strains.

We conclude that meropenem is, *in vitro*, highly active against *N. meningitidis* and *S. pneumoniae* isolates from CSF and may emerge as an effective antimicrobial treatment for patients with bacterial meningitis.

References

1. Enting, R. H., Spanjaard, L., van de Beek, D., Hensen, E. F., de Gans, J. & Dankert, J. (1996). Antibiotic susceptibility of *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* isolates causing meningitis in The Netherlands, 1993–1994. *Journal of Antimicrobial Chemotherapy* **38**, 777–86.

2. Jackson, L. A., Tenover, F. C., Baker, C., Plikaytis, B. D., Reeves, M. W., Stocker, S. A. *et al.* (1994). Prevalence of *Neisseria meningitidis* relatively resistant to penicillin in the United States, 1991. *Journal of Infectious Diseases* **169**, 438–41.

3. Appelbaum, P. C. (1992). Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. *Clinical Infectious Diseases* **15**, 77–83.

4. Jones, D. M. & Sutcliffe, E. M. (1990). Meningococci with reduced susceptibility to penicillin. *Lancet* **335**, 863–4.

5. Woods, C. R., Smith, A. L., Wasilauskas, B. L., Campos, J. & Givner, L. B. (1994). Invasive disease caused by *Neisseria meningitidis* relatively resistant to penicillin in North Carolina. *Journal of Infectious Diseases* **170**, 453–6.

6. Sáez-Nieto, J. A., Lujan, R., Berrón, S., Campos, J., Viñas, M., Fusté, C. *et al.* (1992). Epidemiology and molecular basis of penicillin-resistant *Neisseria meningitidis* in Spain: a 5-year history (1985–1989). *Clinical Infectious Diseases* **14**, 394–402.

7. Lister, P. D. (1995). Multiply-resistant pneumococcus: therapeutic problems in the management of serious infections. *European Journal of Clinical Microbiology & Infectious Diseases, Suppl. 1*, 18–25.

8. Barakett, V., Lesage, F., Delisle, F., Richard, G. & Petit, J. C. (1996). Killing kinetics of meropenem against penicillin-resistant pneumococci. *Journal of Antimicrobial Chemotherapy* **38**, 1085–9.

9. Klugman, K. P. & Dagan, R. (1995). Carbapenem treatment of meningitis. *Scandinavian Journal of Infectious Diseases, Suppl. 96*, 45–8.

10. National Committee for Clinical Laboratory Standards. (1993). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Third Edition: Approved Standard M7-A3.* NCCLS, Villanova, PA.