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Enting, R.H.; Spanjaard, L.; van de Beek, D.; Hensen, E.F.; de Gans, J.; Dankert, J.

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Antimicrobial susceptibility of *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* isolates causing meningitis in The Netherlands, 1993–1994

R. H. Enting[a], L. Spanjaard[b], D. van de Beek[a], E. F. Hensen[a], J. de Gans[a] and J. Dankert[b]

[a]Department of Neurology H2-214, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam; [b]The Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam, The Netherlands

The increasing antimicrobial resistance among pathogens frequently isolated from patients with bacterial meningitis formed the rationale to perform a surveillance study to determine the prevalence of resistance in The Netherlands. *Haemophilus influenzae* strains ($n = 316$) isolated from cerebrospinal fluid (CSF), 1125 meningococcal strains isolated from blood or CSF and 398 pneumococcal strains isolated from CSF in 1993 and 1994 were tested by the Etest for susceptibility to commonly prescribed antibiotics for the treatment of community-acquired meningitis. In *H. influenzae* strains ampicillin-resistance occurred in 7.0%, resistance to chloramphenicol in 2.2%, and resistance to both antibiotics in 0.9%. The prevalence of intermediate penicillin-resistance in meningococci was 3.3%. Resistance to rifampicin was rarely found (0.1%). Intermediate penicillin-resistance in pneumococci was found in only 0.5% of isolates. All 1839 isolates were susceptible to ceftriaxone. Based on these results, we conclude that empirical therapy of childhood community-acquired bacterial meningitis with amoxycillin and chloramphenicol is no longer justified in children who have not been vaccinated against *H. influenzae* type b. In vaccinated or older children and adults, amoxycillin is a rational choice for empirical treatment of meningitis. The prophylactic use of rifampicin in contacts of patients with meningococcal disease is still applicable.

Introduction

The annual incidence of bacterial meningitis in The Netherlands is estimated to be 7–8 cases per 100,000 persons. The three most common bacterial pathogens causing community-acquired meningitis are *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae*, being responsible for 95% of all cases. The incidence of *H. influenzae* type b meningitis in The Netherlands has decreased since vaccination against this bacterium was introduced in July 1993 (Netherlands Reference Laboratory for Bacterial Meningitis (RIVM/UvA), 1994, 1995).

The empirical treatment of acute bacterial meningitis is based on the anticipated bacterial species in each age group and clinical setting, the antibiotic susceptibility of the infecting organism, the achievement of bactericidal concentrations in the CSF, and

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the potential side effects. Guidelines for initial treatment of community-acquired bacterial meningitis in The Netherlands recommend amoxycillin and chloramphenicol in the age group 4 months–10 years, and penicillin or amoxycillin in older children or adults, until the results of culture and susceptibility-testing are known. In case of susceptible *N. meningitidis* or *S. pneumoniae* isolates, benzylpenicillin alone is recommended, and for amoxycillin-susceptible *H. influenzae* chloramphenicol is discontinued. Cephalosporins have become a useful alternative (Roord, 1989). These Dutch guidelines are comparable with those in the UK (Lambert, 1994).

Surveillance studies are important to monitor changing patterns of resistance. Conventional methods of determining the MIC of isolates are time-consuming, but a new method, the Etest, which combines disc diffusion and agar dilution principles is simple and accurate (Jorgensen, Howell & Maher, 1991; Hughes *et al.*, 1993; Jorgensen *et al.*, 1994). In order to update guidelines for the empirical treatment of patients with community-acquired bacterial meningitis over the age of 4 months in The Netherlands, we determined the MICs of antibiotics for the three predominant pathogens. Rifampicin is recommended for chemoprophylaxis of meningococcal disease and MICs of this antibiotic were also determined (Roord, 1989).

Materials and methods

Bacterial strains and growth conditions

The Netherlands Reference Laboratory for Bacterial Meningitis of the University of Amsterdam and the National Institute of Public Health and Environmental Protection (RIVM) in Amsterdam collects strains causing meningitis across the country. In 1993 and 1994, the Reference Laboratory received 1765 strains isolated from CSF and/or blood from patients with bacterial meningitis (316 *H. influenzae*, 830 *N. meningitidis*, 398 *S. pneumoniae*, and 221 miscellaneous bacteria).

We tested all CSF isolates from patients with bacterial meningitis caused by the three predominant pathogens. Whenever a particular CSF isolate was no longer available for testing, the blood isolate from the same patient was taken as a substitute. Furthermore, we tested all 302 *N. meningitidis* strains isolated from blood only. We considered all patients with meningococcal disease important for the purpose of this study, as it is often difficult to make a distinction between septicaemia and meningitis. In Table I the number of isolates obtained per year and age categories of patients are given.

Table I. Source of tested isolates and age category of patients (number of isolates)

Specimen by year	*H. influenzae*	*N. meningitidis*	*S. pneumoniae*
CSF			
1993	202	484	203
1994	114	348	195
blood			
1993	—	154	—
1994	—	139	—
patient age (years)			
0–4	290	354	106
5–16	11	513	40
>17	15	258	252

The identity of all isolates was confirmed upon receipt using standard procedures. Isolates were stored at −70°C in glycerol-based medium on plastic beads. One or two beads were removed from stock cultures, and subcultured onto chocolate blood agar plates (*H. influenzae* and *N. meningitidis*) or blood agar plates (*S. pneumoniae*) and incubated for 18–24 h at 35°C in air with 5% CO_2. Quality control (QC) strains used in this study were *Escherichia coli* (ATCC 25922, *Staphylococcus aureus* ATCC 29213, *H. influenzae* ATCC 49247 and *S. pneumoniae* ATCC 49619, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). No *N. meningitidis* QC strain is available. QC testing was performed daily for 30 days and then weekly.

Serogroup and/or serotype determination

Serotyping of *H. influenzae* isolates was performed by co-agglutination using in-house raised polyclonal rabbit antisera (Campos, 1995). Serogrouping of meningococci was performed by immunodiffusion using in-house raised polyclonal rabbit antisera (Slaterus, 1961). Serotyping was done by whole cell ELISA using a set of seven monoclonal antibodies, and subtyping by using a set of 12 monoclonal antibodies obtained from the RIVM (Bilthoven, The Netherlands) (Abdillahi & Poolman, 1987). Serotyping of *S. pneumoniae* isolates was performed by the capsular swelling method (Quellung reaction) with antisera obtained from the Statens Serum Institut (Copenhagen, Denmark).

Susceptibility testing

In the Reference Laboratory, β-lactamase production by *H. influenzae* strains is determined upon the receipt of a strain by the nitrocephin assay. Penicillin-susceptibility of meningococci is determined by inoculating strains on chocolate agar containing 0.1 mg/L penicillin. Penicillin-resistance in pneumococci is determined using a 1 μg oxacillin disc.

Etest strips were provided by AB Biodisk (Solna, Sweden). The inoculation procedure described by NCCLS (1993) was used. Briefly, inocula were prepared by suspending bacteria in phosphate-buffered saline to achieve a turbidity equivalent to 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 *H. influenzae* we used Haemophilus Test Medium agar, for meningococci 5% chocolate Mueller-Hinton agar, and for *S. pneumoniae* strains Mueller-Hinton 5%-sheep blood agar. All plates were incubated for 18–24 h at 35°C in 5% CO_2. The MIC value was read where bacterial growth intersected the Etest strip. QC strains were tested in the same way. For calculation of the MIC_{50} and the MIC_{90} the actual MICs as determined by the Etest were used. For determination of the susceptibility category (susceptible/intermediately resistant/resistant), the Etest results were rounded up to the nearest two-fold dilution value as recommended by the manufacturer.

Choice of antimicrobial agents and evaluation criteria

The choice of antibiotics was determined by the current treatment regimens used in The Netherlands for meningitis and can be found in Table II (Roord, 1989). Antimicrobial

Table II. Criteria for susceptibility test interpretation

	Susceptible	Intermediate resistant	Resistant
H. influenzae			
ampicillin	≤1	2	≥4
ceftriaxone	≤2		
chloramphenicol	≤2	4	≥8
N. meningitidis			
penicillin	≤0.06	0.1–1	≥2
ceftriaxone	≤0.25	0.5–1	≥2
chloramphenicol	≤2	4	≥8
rifampicin	≤1	2	≥4
S. pneumoniae			
penicillin	≤0.06	0.1–1	≥2
ceftriaxone	≤0.25	0.5–1	≥2
chloramphenicol	≤4	8	≥16

agent concentrations ranged 0.016–256 mg/L for chloramphenicol and ampicillin and 0.002–32 mg/L for penicillin, rifampicin and ceftriaxone.

The MIC criteria used for the Etest were those used for microorganisms tested by dilution susceptibility test methods (NCCLS, 1993) (Table II). For meningococcal isolates, criteria for susceptibility to penicillin and rifampicin have been suggested elsewhere (Jones & Sutcliffe, 1990; Jackson *et al.*, 1994), and results for chloramphenicol and ceftriaxone were interpreted according to the strictest NCCLS (1993) criteria for other microorganisms.

Statistical analysis

The following data were available: year and month of isolation, hospital, site of isolation, age and sex of patient, organism serogroup/serotype and susceptibility pattern to penicillin and β-lactamase production. Medians were calculated using the Statistical Package for the Social Sciences (SPSS) 6.0 for Windows and comparisons of proportions were calculated using CIA (Confidence Interval Analysis) (Gardner & Altman, 1989).

Results

Susceptibility testing

Seven meningococcal strains were either lost or could not be subcultured. All other strains were available for testing. Routine susceptibility testing upon receipt of the isolates revealed that 30 (9.5%) *H. influenzae* strains produced β-lactamase and 10 (0.9%) meningococcal strains had intermediate susceptibility to penicillin. No penicillin-resistant (1.0 μg oxacillin disc) pneumococci were isolated. By Etest results, 7% of *H. influenzae* isolates were resistant to ampicillin, 2.2% to chloramphenicol and 1% to both (Table III). *N. meningitidis* isolates (Table IV) were intermediately resistant to penicillin in 3.3% of cases. The only blood isolate which was highly resistant (MIC > 256 mg/L) to rifampicin was susceptible to penicillin. Resistance among *S. pneumoniae* isolates (Table V) occurred in less than 1%.

Table III. Antimicrobial susceptibility of 316 CSF isolates of *H. influenzae* (1993–1994) as determined by Etest

Antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Number (%) of resistant strains intermediate	high
Ampicillin	0.19	0.25	20 (6.3)	2 (0.6)
children[a]	0.19	0.38	20 (6.6)	2 (0.7)
adults	0.19	0.25		
Ceftriaxone	0.003	0.003		
Chloramphenicol	0.38	0.38	7 (2.2)	
children[b]	0.38	0.38	7 (2.3)	
adults	0.25	0.38		

[a]Mean difference (MD) of prevalence of ampicillin resistance between children and adults is 7.3% (95% CI 4.4–10.2%).

[b]MD of prevalence of chloramphenicol resistance between children and adults is 2.3% (95% CI 0.6–4.0%).

A discrepancy between routine susceptibility testing and the Etest was found in ten *H. influenzae* strains: one β-lactamase negative strain was found to be ampicillin-resistant and 9/30 β-lactamase producing strains had Etest MICs of ampicillin in the susceptible range. As different criteria were used for the routine testing of meningococci and pneumococci, results cannot be similarly compared with the Etest.

The distributions of Etest MICs of penicillin, ampicillin and chloramphenicol for the appropriate bacteria are shown in Figure (a)–(c), respectively.

Antibiotic susceptibility according to serogroup and serotype

Twenty-two of 298 *H. influenzae* type b strains (7.4%) were resistant to ampicillin, whilst none of 18 non-type b strains were similarly resistant (mean difference 7.4%, 95% CI: 4.4%–10.4%). The overall *N. meningitidis* distribution showed that 83%, were serogroup B and 15% serogroup C, and of intermediate resistant strains 30 (81%) were serogroup B and 7 (18%) serogroup C. Four strains were identified as B:4:P1.15, while this subtype represents only 4% of all serogroup B cases. The main serotypes of *S. pneumoniae* isolates were serotype 14 and 23F (each 8.9%), type 6B (8.7%), 18C (8.4%) and 19F (8.2%). Intermediate penicillin-resistant strains were of serotype 23F (1/35) and 35B (1/1), and the intermediate chloramphenicol-resistant strain belonged to serogroup 8 (1/9).

Table IV. Antimicrobial susceptibility of 293 blood and 832 CSF isolates of *N. meningitidis* (1993–1994) as determined by Etest

Antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Number (%) of resistant strains intermediate	high
Penicillin	0.032	0.047	37 (3.3)	
Ceftriaxone	<0.002	<0.002		
Chloramphenicol	0.75	1.0		
Rifampicin	0.012	0.032		1 (0.1)

Table V. Antimicrobial susceptibility of 398 CSF isolates of *S. pneumoniae* (1993–1994) as determined by Etest

Antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Number (%) of resistant strains intermediate	high
Penicillin	0.016	0.023	2 (0.5)	
Ceftriaxone	0.016	0.023		
Chloramphenicol	1.5	3.0	1 (0.3)	

Antibiotic susceptibility according to source

CSF isolates of *N. meningitidis* were penicillin resistant in 2.9% of cases as compared with 4.4% of blood isolates. ($P > 0.05$) One blood isolate was highly resistant to rifampicin. MIC_{50}s of all tested antibiotics were identical for blood and CSF isolates (data not shown).

Antibiotic susceptibility according to age of patients

H. influenzae isolates from children were significantly more often ampicillin resistant compared with those from adults (Table III). All resistant strains were isolated from children ≤ 4 years of age. For *N. meningitidis* isolates, no age differences in the prevalence of antibiotic resistance were found. MIC_{50}s of all antibiotics were identical for both age groups (data not shown).

Antibiotic susceptibility according to year of receipt

No significant differences were found in the percentages of resistant strains from 1993 compared with 1994.

Discussion

Ampicillin resistance was found in 7.0% of *H. influenzae* isolates according to the Etest and 9.5% according to β-lactamase production. Resistance to chloramphenicol was noted in 2.2% of isolates. Resistance to chloramphenicol was associated with resistance to ampicillin in 0.9% of strains. These findings are similar to prevalences among isolates from different types of specimen in nine European countries (1988–89) and South Africa (1991–92) (Kayser, Morenzoni & Santanam, 1990; Hussey *et al.*, 1994). In the USA ampicillin resistance in *H. influenzae* occurred twice as often, yet chloramphenicol resistance was rare (Doern *et al.*, 1988). A significantly higher prevalence of ampicillin resistance was found among our type b as compared with non-type b strains. This was also seen in the USA, but not in previous studies from Europe and South Africa (Doern *et al.*, 1988; Kayser *et al.*, 1990; Hussey *et al.*, 1994). One study mentioned highest rates of ampicillin resistance among children aged less than 5 years, comparable to our findings (Doern *et al.*, 1988). As the outcome of patients with *H. influenzae* meningitis caused by strains which were resistant to initial therapy consisting of ampicillin and chloramphenicol appeared to be worse in one study, this regimen cannot be recommended now for *H. influenzae* meningitis in The Netherlands (Campos *et al.*, 1986).

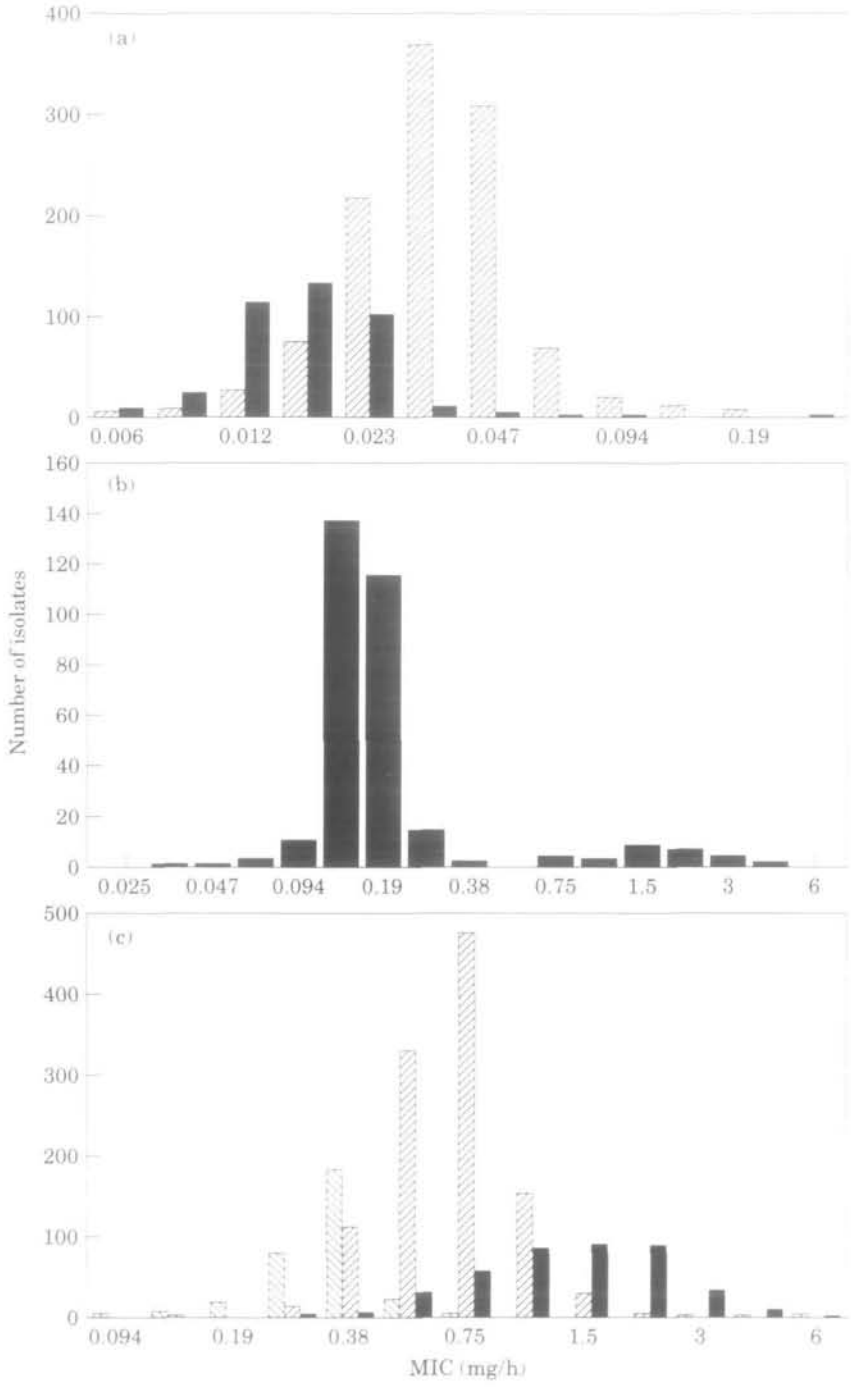

Figure. Distributions of MICs of penicillin (a), ampicillin (b) and chloramphenicol (c) for appropriate meningitis pathogens in The Netherlands (1993–94). (a) ▨, *N. meningitidis* ($n = 1125$); ■, *S. pneumoniae* ($n = 398$); (b) ■, *H. influenzae*, ($n = 316$); (c) ▧, *H. influenzae* ($n = 316$); ▨, *N. meningitidis* ($n = 1125$), ■, *S. pneumoniae* ($n = 398$).

Analysis of our *N. meningitidis* isolates revealed an overall prevalence of intermediate penicillin resistance of 3.3%, comparable to prevalences found in the UK (1980s), USA (1991), and Canada (1991–1992), but far lower than the 20–40% prevalence in Spain (1989–1990) (Jones & Sutcliffe, 1990; Saez-Nieto *et al.*, 1992; Jackson *et al.*, 1994; Ringuette *et al.*, 1995). Among our intermediately resistant strains, serogroup B:4:P1.15 was relatively often present, as found in Spain (Saez-Nieto *et al.*, 1992). Penicillin is still regarded as first choice for the treatment of meningococcal meningitis with intermediate resistant strains (Jones & Sutcliffe, 1990). Secondary cases caused by failure of rifampicin chemoprophylaxis have been reported (Yagupsky, Ashkenazi & Block, 1993), but as only 0.1% of our strains were rifampicin resistant, chemoprophylaxis with this antibiotic is still applicable in The Netherlands.

Resistance to penicillin or chloramphenicol in *S. pneumoniae* CSF isolates was very rare. With the recently suggested change in Etest (penicillin) susceptibility categories for pneumococci, 6 (1.5%) strains would be classified as intermediately resistant, and none as resistant (Scheel *et al.*, 1995). The rare occurrence of resistance cannot be explained by a different serodistribution compared with other regions, as the four serogroups known for high rates of resistance (6, 14, 19, 23) represent 40% of CSF isolates in The Netherlands (Appelbaum, 1992; Netherlands Reference Laboratory for Bacterial Meningitis (RIVM/UvA), 1994). A more likely explanation is that in The Netherlands antibiotics are available by prescription only, and guidelines recommending restricted use of antibiotics in various bacterial infections are widely used.

In fact, the prevalence of resistance found in our pneumococcal CSF isolates is among the lowest in the world. In adjacent countries, the prevalences of penicillin resistance in CSF and blood isolates were 4.3% (Belgium, 1986–1993) and 1.8% (Germany, 1992–94) as compared with 0.5% in our isolates. Chloramphenicol resistance was found in 2.7% and 1.9% of isolates from Belgium and Germany, respectively, as compared with 0.3% of isolates in this study (Reinert *et al.*, 1995; Verhaegen *et al.*, 1995). The prevalence of penicillin resistance in CSF isolates was 40% and 25% in Spain (1979–89) and France (1993), respectively; 25% of isolates were chloramphenicol resistant (Fenoll *et al.*, 1991; Linares *et al.*, 1992; Olivier *et al.*, 1994). In a recent population-based surveillance of invasive pneumococcal infections in the USA 25% of isolates were resistant to penicillin and 3% to chloramphenicol. (Hofmann *et al.*, 1995). The findings in the present study do not justify a change in initial therapy of presumptive pneumococcal meningitis.

We found a disturbingly high discrepancy (30%) between the results of the β-lactamase assay and ampicillin Etest in β-lactamase producing *H. influenzae* strains. One study mentioned a 7% prevalence of discrepancy between MIC results and the nitrocephin assay among resistant strains (Kayser *et al.*, 1990). A discrepancy is often explained by the fact that β-lactamase producing strains yield inhibition ellipses which may be difficult to interpret (Jorgensen *et al.*, 1991). After evaluation of our observed Etest results, we found that by using a tentative cut-off MIC of 0.75 mg/L, only 2/30 β-lactamase-positive strains would have been missed, while only one more strain would have been classified as β-lactamase-negative ampicillin-resistant.

Several conclusions may be drawn regarding empirical therapy in bacterial meningitis. In childhood (4 months–4 years) bacterial meningitis in The Netherlands a distinction should be made between children who have been vaccinated with the *H. influenzae* type b vaccine and children who have not been immunized. In the first group, only sporadic cases of non-type b *H. influenzae* meningitis will occur. As no resistant

non-type b isolates were found in this study, amoxycillin monotherapy may become the first-line treatment in vaccinated children. For non-vaccinated children, a switch to a third generation cephalosporin is warranted because *H. influenzae* type b meningitis caused by strains resistant to both compounds of standard treatment may occur.

Bacterial meningitis in older children (≥ 5 years) and adults caused by *H. influenzae* occurs in less than 3% of cases and no resistant CSF isolates were detected in patients over the age of 4 years. On the other hand, meningitis due to *Listeria monocytogenes* occurs in about 2% and this bacterium is resistant to cephalosporins (Netherlands Reference Laboratory for Bacterial Meningitis (RIVM/UvA), 1994, 1995). Therefore, empirical therapy with amoxycillin or penicillin, and not a cephalosporin, is preferable in community-acquired bacterial meningitis in older children or adults.

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