CONCISE COMMUNICATION

Susceptibility of pneumococci to evernimicin: effect of CO_2 and different methodologies

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Accurate determination of the antibiotic susceptibility of pathogens is essential for both patient management and epidemiologic purposes. Unfortunately, there are differences in methodology (e.g. media, inoculum, incubation conditions) and interpretation (e.g. numerical values of breakpoint concentrations and zone sizes) between recommendations approved by various organizations. Results using different methods have not been correlated for all antibiotics.

Such differences appear to be of particular importance in the case of pneumococci, as incubation in the presence of CO_2 is recommended by some but not all testing protocols. For example, incubation in CO_2 is recommended by the NCCLS and BSAC for agar diffusion [1,2], by the Swedish guidelines [3] and for the E test [4]. On the other hand, incubation in air is suggested by the NCCLS for broth dilution [5], and by BSAC for agar dilution [6]. The European Committee [7] advises the use of CO_2 only when 'necessary', while French guidelines [8] do not address this problem.

CO_2 is a well recognized confounding factor in susceptibility testing, as, among other factors, the reduction in pH it engenders may significantly alter the apparent activity of some antibiotics. Thus, ideally it should be used routinely only when absolutely necessary for the growth of the organism being tested (e.g. for gonococci), which, notwithstanding the recommendations mentioned above, is not the case for pneumococci. We have recently found (unpublished) that only six of 175 freshly isolated strains of pneumococci (3.4%) were strictly capnophilic.

We wish to illustrate discrepancies that can arise between results obtained using different methods, giving as an example comparative data recently obtained with evernimicin, a member of the orthosomycin family of polysaccharide antibiotics [9]. MICs determined by plate dilution were compared with results of E tests, using different media and incubation conditions, as recommended by the NCCLS, the BSAC and AB Biodisk (the manufacturers of the E test). Evernimicin has been shown to diffuse too poorly to be tested by the standard disk diffusion method [10].

MICs of evernimicin (SCH 27899, kindly supplied by the Schering Plough Research Institute) against 50 clinical isolates of *Streptococcus pneumoniae* were determined by four different methods: agar dilution, according to NCCLS and BSAC recommendations, and E test using Mueller–Hinton agar (MHA) and IsoSensitest agar (ISA). In addition, the effect of CO_2 on the activity of evernimicin was investigated by testing 20 of the strains on MHA incubated in CO_2 (i.e. standard NCCLS conditions) and in air. Results of E tests were read using an endpoint of 80% inhibition, as recommended by Marshall et al [11]. Media were obtained from Unipath Ltd, Basingstoke, UK. The control strain was *S. pneumoniae* ATCC 49619, as recommended by the NCCLS [5].

Table 1 shows that MICs obtained using the BSAC method were almost double those determined according to the NCCLS protocol. This difference can be explained by the effect of CO_2—present in the test conducted following NCCLS guidelines, absent when using the BSAC method. From Table 2 it can be seen that under otherwise identical conditions, incubation in CO_2 causes a two-fold reduction in the MIC of evernimicin.

Marshall et al [11] and Jones and Barrett [12] have, respectively, shown that MICs for pneumococci in MH medium incubated in CO_2 are virtually identical in broth and agar, and our results (Table 1) agree very closely with those of the latter workers.

The results of E tests in the different media (both carried out in the presence of CO_2) were almost identical, but consistently lower than the respective results obtained by the dilution method. A partial explanation of this may be that, as recommended, E test endpoints were read at 80% inhibition, whereas the plate MIC was read, as customary, at 100% inhibition. However, there was little or no discrepancy between E test and plate dilution results when staphylococci and enterococci were tested in a similar manner (unpublished results), so this may be a phenomenon peculiar to pneumococci.

It is not clear which of the MIC values obtained is 'correct' in terms of prediction of therapeutic efficacy. The results here

Table1 Activity of evernimicin against 50 strains of *Streptococcus pneumoniae* and control strain determined by four methods

		MIC (mg/L)				
Medium	**Method**	**Range**	**MIC_{50}**	**MIC_{90}**	**Geometric mean**	**Control strain[a]**
Mueller–Hinton agar	Dilution	0.03–0.13	0.13	0.13	0.09	0.06, 0.06, 0.06
	E test	0.016–0.064	0.032	0.047	0.031	0.047, 0.047, 0.047
IsoSensitest agar	Dilution	0.06–0.25	0.13	0.25	0.17	0.13, 0.13, 0.13
	E test	0.016–0.13	0.032	0.064	0.036	0.047, 0.047, 0.032

[a]*S. pneumoniae* Ascc 49619.

Table 2 Activity of evernimicin against 20 strains of *Streptococcus pneumoniae*: effect of incubation in carbon dioxide on Mueller–Hinton agar

	MIC (mg/L)			
Incubation	**Range**	**MIC_{50}**	**MIC_{90}**	**Geometric mean**
Air	0.13–0.25	0.13	0.25	0.16
Carbon dioxide	0.03–0.13	0.06	0.13	0.068

indicate that testing pneumococci in the presence of CO_2 will overestimate the activity of evernimicin under normocapnic conditions. Although the difference observed was only small (2.4-fold in terms of the geometric mean Table 2), it may be of relevance when considering therapy for, on the one hand, pneumococcal pneumonia (where there will be hypercapnosis), and, on the other hand, otitis media or meningitis, where there is likely to be normocapnosis. There is clear evidence in the case of aminoglycosides, for example, that decreased activity in the presence of CO_2 explains the need to use a larger dose when treating chest infections [13].

We have previously found that CO_2 alters the activity of another novel antibiotic, linezolid, only against pneumococci [14]. In view of this and the findings described above, it would seem appropriate to include comparative studies of different susceptibility testing methodologies at an early stage in the assessment of a novel antibiotic.

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