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Susceptibility testing of *Haemophilus influenzae* to clarithromycin

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Susceptibility testing of *Haemophilus influenzae* to the macrolide compounds has often been problematic. This is as a result of the inability of many isolates of *H. influenzae* to grow without the addition of 5% CO_2 to the incubation atmosphere and the subsequent detrimental effect that CO_2 has on the activity of the macrolide group of compounds. This report describes refinements and recommendations for susceptibility testing of *H. influenzae* to the macrolide clarithromycin.

Introduction

Clarithromycin is effective in patients with infections caused by *Haemophilus influenzae*.[1–3] This is thought to be as a result of the activity of the 14-hydroxy metabolite of clarithromycin exerting an additive or possible synergic effect with the parent compound.[3] Clarithromycin has been widely used for the treatment of respiratory infections. Susceptibility of *H. influenzae* to clarithromycin has been a difficult value to assess, because, like other macrolides, clarithromycin is affected by media composition and atmosphere.[4,5] This makes the selection of an *in vitro* breakpoint for *H. influenzae* problematic. In this report we have investigated the difference in the results of susceptibility testing of *H. influenzae* to clarithromycin using differing disc concentrations and atmospheric conditions.

Materials and methods

Bacterial isolates

One hundred consecutively obtained isolates of *H. influenzae* from respiratory sources were collected from specimens received in the clinical bacteriology laboratories of Edinburgh Royal Infirmary. Isolates were identified as *H. influenzae* by their requirement for XV factors by use of Mast ID rings (Mast Group Ltd, Liverpool, UK). Isolates were stored at –70°C on Microbank beads (Pro-Lab Diagnostics, Neston, UK) until required.

Sensitivity tests

MICs of clarithromycin were determined for all isolates using Etest strips (AB Biodisk, Solna, Sweden) on Iso-Sensitest agar (IST; Oxoid Ltd, Basingstoke, UK) supplemented with 5% horse blood (E & O Laboratories, Stirling, UK) and nicotinamide–adenine dinucleotide (NAD) 20 mg/L (Sigma Chemicals, Dorset, UK). The MIC was defined as the point of intersection between the ellipse edge and the Etest strip where there was complete inhibition of all growth. MICs were determined both in air and in 4–6% CO_2 with incubation overnight at 37°C. Breakpoint values for the scattergrams were extrapolated from the MIC results. Results for isolates incubated in air and CO_2 were therefore set at 8 and 16 mg/L, respectively.

For disc testing two disc concentrations were used, 5 μg and 15 μg (Mast Group Ltd). The same medium was used as for MIC testing. The plates were inoculated with a cotton swab using a rotary plater (Denley Instruments Ltd, Billingshurst, UK) with 0.5 McFarlane standard suspensions of *H. influenzae* diluted 1:100 with sterile distilled water. Immediately after application of the antibiotic discs all isolates were incubated in both air and 4–6% CO_2 overnight. Zone diameters for the disc susceptibility tests were well defined with the technique employed and were measured with a ruler. *Staphylococcus aureus* NCTC 6571 was used as a control; all its values were within the expected limits.

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Results

Results for MIC values and zone diameters for 15 μg discs are shown as scattergrams (Figures 1 and 2). Scattergrams were also constructed for results of MIC values and zone diameters for 2 μg discs (data not shown). Cut-off zones indicated in the charts were chosen to reflect the values that we felt were most practical for routine use. The scattergram results demonstrated that using a cut-off zone of ⩾10 mm diameter with 5 μg discs incubated in CO_2 gave a false resistance rate of 28%, whereas tests with a 15 μg disc incubated in CO_2 exhibited no false resistant results. Twenty-four per cent of the isolates failed to grow without incubation in CO_2. Incubation in air with a 15 μg disc showed no false resistance whereas a 5 μg disc in air gave 10.5% false resistance.

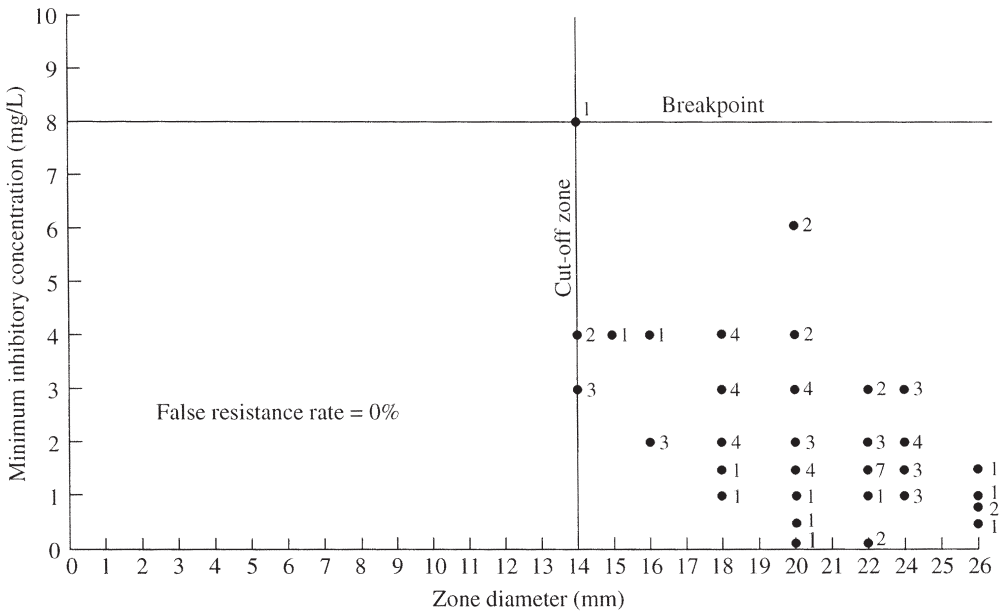

Figure 1. Scattergram showing the susceptibility testing of 76 isolates of *H. influenzae* to clarithromycin (15 μg disc) incubated in air.

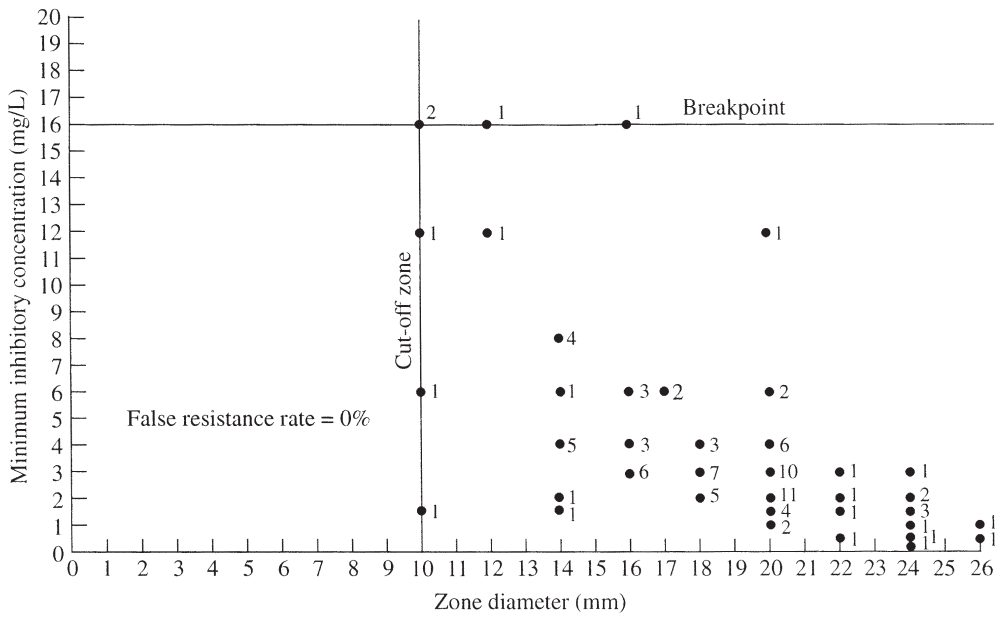

Figure 2. Scattergram showing the susceptibility testing of 100 isolates of *H. influenzae* to clarithromycin (15 μg disc) incubated in CO_2.

Discussion

In our laboratory we formerly tested respiratory isolates of *H. influenzae* for susceptibility to clarithromycin by agar breakpoint method. A breakpoint value of 16.0 mg/L was used and incubation was in 4–6% CO_2 incubated overnight. This value is thought to reflect concentrations of clarithromycin at sites of respiratory infection rather than in serum.[1] The resistance rate determined by this method was <5% during the period 1996–1998.

We have adopted the recent BSAC (British Society for Antimicrobial Chemotherapy) guidelines for disc susceptibility testing of *H. influenzae*.[6] Employing this method it was found that susceptibility testing with clarithromycin employing IST agar with added NAD at 20 mg/L and a 5 μg disc incubated in 4–6% CO_2 overnight increased our resistance rate to approximately 25%.

The BSAC guidelines suggest that clarithromycin MIC values of 1–16 mg/L be categorized as intermediate susceptibility to this drug, with values below and above this range denoting susceptibility and resistance, respectively. The zone diameter for disc susceptibility testing in 4–6% CO_2 overnight suggests a diameter of 10–24 mm denoting intermediate sensitivity, with values below and above this range suggesting sensitivity and resistance, respectively. It should be noted, however, that these guidelines for clarithromycin are tentative and have not been confirmed by a BSAC field trial.

Etest strips were used as the reference method for our study as they have been shown to be reliable for macrolides and have good correlation with the NCCLS reference microdilution method.[5]

The most accurate results in the study were with the 15 μg disc incubated overnight in air; however, this technique suffered from the inability of 24% of the isolates to grow without incubation in CO_2. Testing under these conditions would suggest a cut-off zone of ⩾14 mm diameter to indicate susceptibility. This would greatly aid interpretation of the results. The ⩾10 mm zone diameter recommended at present with a 5 μg disc incubated in 4–6% CO_2 is only 4 mm larger than the disc size itself and is a difficult value to measure. Clearly, these results show that the use of a 5 μg disc is unacceptable whether incubated in air or CO_2. Results obtained with a 15 μg disc incubated in CO_2 would indicate that this method was the most practical for day-to-day use. A cut-off zone of ⩾10 mm diameter denoting susceptibility would still be indicated for use but only 5% of isolates tested gave this value; most were ⩾14 mm diameter.

It is important that we address problems of susceptibility testing of any organism on a dynamic continuing basis and that reports to clinicians reflect the *in vivo* response as accurately as possible. We would therefore recommend that for routine susceptibility testing of *H. influenzae* from respiratory sources a 15 μg disc be used. Incubation should be in 4–6% CO_2, a zone diameter of ⩾10 mm denoting sensitivity and ⩽9 mm diameter denoting resistance. If the breakpoint method is used the value should be 16 mg/L with incubation in CO_2 overnight.

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