Journal of Antimicrobial Chemotherapy (1999) **43**, 141–143

JAC

Study to assess the reliability of a disc diffusion method for determining the sensitivity of Gram-positive pathogens to dalfopristin/quinupristin

J. M. Andrews, J. P. Ashby and R. Wise

Department of Medical Microbiology, City Hospital NHS Trust, Birmingham B18 7QH, UK

A standardized method of disc testing the sensitivity of Gram-positive pathogens to dalfopristin/quinupristin was developed, and then 'field tested' in ten centres in the UK. For a 15 μg disc, zone diameter breakpoints of 20 mm and 15 mm are suggested when organisms are tested on Iso-Sensitest agar and Iso-Sensitest agar supplemented with 5% whole horse blood, respectively.

Introduction

Dalfopristin/quinupristin (Synercid), in a 70:30 ratio, is a new streptogramin with activity against Gram-positive pathogens, including those strains known to be resistant to methicillin, vancomycin, erythromycin and clindamycin.[1] Using procedures appropriate to the UK and used in a modified form in other European countries, namely a semi-defined medium and an inoculum equivalent to semi-confluent growth, a standardized method of disc testing was developed for use in diagnostic laboratories. Following this, it was then considered prudent to 'field test' the reliability of the method in centres located in the UK.

Materials and methods

Method development

Recent clinical isolates (n = 4732) from hospitals in the UK, comprising 1745 *Staphylococcus aureus,* 1381 coagulase-negative staphylococci (CNS), 1040 *Streptococ - cus pneumoniae* and 566 enterococci, and control strains *S. aureus* NCTC 6571 and ATCC 25923, *S. pneumoniae* ATCC 49619 and *Enterococcus faecalis* ATCC 29212 were studied.

Field testing study

Fifty strains, comprising 23 staphylococci, 17 enterococci and ten pneumococci, with MICs of dalfopristin/quinupristin ranging from 0.06 mg/L to 4 mg/L, were distributed by a central laboratory to ten centres in the UK. Included among these organisms were the four control strains mentioned previously.

MICs

MICs were determined by the central laboratory using methodology described in 'A Guide to Sensitivity Testing'.[2] Briefly, Iso-Sensitest agar (ISTA; Oxoid, Basingstoke, UK) was used as basal medium. When testing pneumococci, the medium was supplemented with 5% whole horse blood (E and O Laboratories, Bonnybridge, UK). An inoculum of 10^4 cfu was used and incubation was for 18–20 h at 35–37°C in air, except for the pneumococci, where incubation was in an atmosphere enriched with 4–6% CO_2. The MIC was defined as the lowest concentration of dalfopristin/quinupristin inhibiting growth, one or two colonies being ignored.

Disc testing

The field testing laboratories were instructed to use the medium described previously. Agar (20–25 mL) was poured into 90 mm sterile Petri dishes to give a depth of 3–4 mm. Before inoculation, the surface of the agar was dried to remove excess moisture. Organism suspensions or overnight broth cultures were initially adjusted with sterile distilled water to a density equivalent to the 0.5 McFarland standard. A further dilution in sterile distilled water of 1:100 (enterococci) or 1:10 (staphylococci and pneumococci) was made before the organism suspension was swabbed on to the surface of the agar plate. A disc containing 15 μg of dalfopristin/quinupristin was placed on the surface of the agar and the plates were incubated (stacking of plates more than six high in the incubator was avoided) using the atmospheric conditions detailed previously. After incubation, zone diameters (in millimetres) were measured using a ruler.

Determination of sensitivity

The sensitivity of an organism is determined by comparing its MIC with an in-vitro breakpoint concentration: if the MIC is equal to or less than the in-vitro breakpoint the organism is regarded as sensitive.[2] A tentative breakpoint for dalfopristin/quinupristin of 2 mg/L has been suggested by Rhône–Poulenc Rorer based on worldwide MIC distribution data and clinical response data (Nadler, H., Dowzicky, M., Talbot, G., Bompart, F. & Pease, M., Rhône–Poulenc Rorer Pharmaceuticals, Collegeville, PA, USA, personal communication).

Results and discussion

MICs plotted against diameters of zones of inhibition for the clinical isolates and control strains used to develop the standardized method are shown in Figure 1. MICs for the control strains, NCTC 6571 (organism 1), ATCC 25923 (organism 2), ATCC 49619 (organism 41) and ATCC 29212 (organism 32) were 0.12, 0.25, 0.5 and 4 mg/L, respectively (corresponding zones of inhibition were 30, 29, 25 and 16 mm). From these data a zone breakpoint of 20 mm was chosen to separate the sensitive and the resistant populations; isolates with MICs ≤2 mg/L and a zone of inhibition ≥20 mm were regarded as sensitive, while those with MICs ≥4 mg/L and a zone of inhibition ≤19 mm were regarded as resistant. In Figure 2 mean zone diameters ± 1 S.D. for the combined data from the ten centres are shown for each of the 50 distributed strains. Little inter-laboratory variation in zones of inhibition was observed (S.D. range 0.67–3.51 mm). Among the laboratories adhering to the recommended methodology, false reporting was observed with only two strains. The first (organism 40), a *Staphylococcus haemolyticus* isolate with an MIC of 1 mg/L, was reported resistant by one centre; the second (organism 34), an *E. faecalis* isolate with an MIC of 2 mg/L, was reported resistant by all centres. False reporting was observed from two centres modifying the recommended method by the addition of blood to the basal medium when testing three strains of CNS (one strain each of *Staphylococcus cohni*, *Staphylococcus capitis* and *Staphylococcus warneri*) and one strain of *Enterococcus gallinarum*. It was considered by these centres that the growth of these organisms on ISTA alone was not as luxuriant as the growth of the other staphylococci and enterococci tested. Zones of inhibition were considerably smaller on ISTA supplemented with blood than those obtained on ISTA alone; for example, a zone of 19 mm was obtained for the *S. cohni* on supplemented ISTA compared with 27 mm on ISTA alone, and zones of inhibition for the *E. gallinarum* were reduced from 23 mm on ISTA alone to 13 mm on ISTA supplemented with blood. In both of these instances, these strains with MICs ≤1 mg/L would have been falsely interpreted as resistant to dalfopristin/quinupristin on ISTA supplemented with blood.

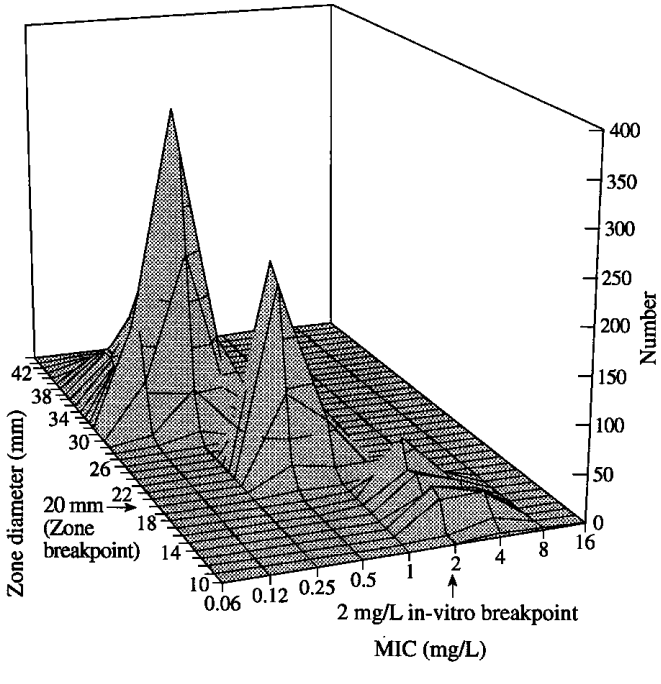

Figure 1. Three-dimensional graph of number of isolates, MIC and zone of inhibition (15 μg disc) for 4732 clinical isolates and control strains to dalfopristin/quinupristin. A 20 mm zone diameter breakpoint and an in-vitro breakpoint of 2 mg/L separate the sensitive and resistant populations.

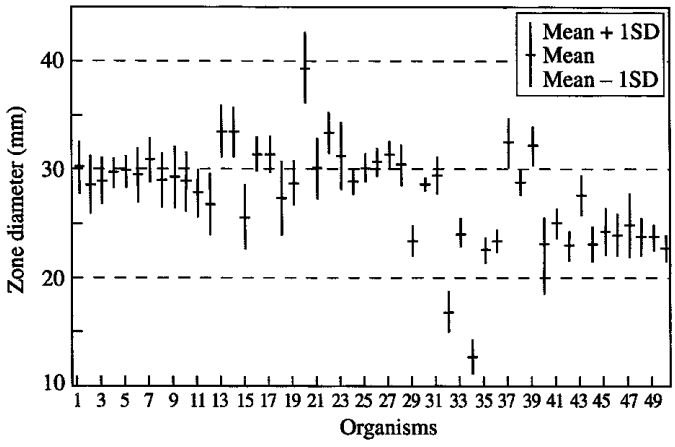

Figure 2. Plot of mean zone diameters ± 1 S.D. for the 50 strains tested by ten centres in the UK. Organisms 1–10 are *S. aureus* strains, 11–23 CNS, 24–40 enterococci and 41–50 pneumococci.

Although not fully explained, the presence of blood has a marked effect on the in-vitro activity of dalfopristin/quinupristin.[3] Indeed, the zones of inhibition for the control strains used in this study were reduced in the presence of blood (zones of inhibition for NCTC 6571, ATCC 25923 and ATCC 29212 when tested on ISTA with the addition of 5% whole horse blood zones of inhibition were 26, 22 and 11 mm, respectively). In the rare situation where blood needs to be added to enhance the growth of

staphylococci or enterococci, the interpretative zone for sensitivity should be reduced to 15 mm.

In conclusion, this study has shown that reproducible disc susceptibility testing is possible for dalfopristin/quinupristin. The problem of the effect of blood upon the laboratory methodology has been highlighted.

Acknowledgements

We would like to thank the following: Professor I. Phillips, St Thomas' Hospital, London; Dr D. Brown, Addenbrookes Hospital, Cambridge; Dr A. Quoraishi, Llandough Hospital, Cardiff; Dr R. Spencer, PHL, Bristol Royal Infirmary, Bristol; Dr A. Swann, PHL, Leicester Royal Infirmary, Leicester; Dr F. Emmanuel, University of Edinburgh, Edinburgh; Dr P. Murphy, City Hospital, Belfast; Dr Drabu, North Middlesex Hospital, London; Dr M. Wilcox, University of Leeds, Leeds; Dr Sadiq, PHL, Northern General Hospital, Sheffield. We would also like to thank Rhône–Poulenc Rorer for their financial support.

References

1. Neu, H. C., Chin, N. X. & Gu, J. W. (1992). The in-vitro activity of new streptogramins, RP 59500, RP 57669 and RP 54476, alone and in combination. *Journal of Antimicrobial Chemotherapy* **30**, *Suppl. A*, 83–94.

2. Working Party on Antibiotic Sensitivity of the British Society for Antimicrobial Chemotherapy. (1991). A guide to sensitivity testing. *Journal of Antimicrobial Chemotherapy* **27**, *Suppl. D*, 1–50.

3. Andrews, J. M. & Wise, R. (1994). The in-vitro activity of a new semi-synthetic streptogramin compound, RP 59500, against staphylococci and respiratory pathogens. *Journal of Antimicrobial Chemotherapy* **33**, 849–53.

Received 20 April 1998; returned 17 June 1998; revised 6 July 1998; accepted 12 August 1998