RESEARCH NOTE

Development of a novel assay method for colistin sulphomethate

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ABSTRACT

Increased use of colistin therapy for infections caused by *Pseudomonas aeruginosa* has indicated a need for a more robust microbiological assay technique. This report describes a quick and simple microbiological assay for quantifying levels of colistin sulphomethate in serum and urine samples from cystic fibrosis patients. The technique uses no specialised or costly equipment and is suitable for use in all routine diagnostic microbiology laboratories.

Keywords Assay method, bioassay, colistin, cystic fibrosis, *Pseudomonas aeruginosa*

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Colistin, a multicomponent polypeptide composed mainly of colistin A and B, is an effective treatment of respiratory exacerbations caused by *Pseudomonas aeruginosa* in cystic fibrosis patients [1]. Colistin is available as colistin sulphate, for oral or topical use, and colistin sulphomethate (CMS), for parenteral and inhalation therapy. Both preparations are hydrolysed to a complex mixture of partially sulphonated derivatives, which possess varying antibacterial activities, as well as colistin itself. Previously, intravenous use of colistin was limited by concerns regarding toxicity; however, with increased resistance to other anti-pseudomonal drugs and the lower toxicity of CMS, use of colistin is increasing. Early therapy with inhaled CMS is now being used with good effect to prevent deterioration in cystic fibrosis patients with chronic infection, and a number of chronically infected cystic fibrosis patients have also been treated successfully with 3-month courses of intravenous CMS [1–3]. Although resistance to colistin is rare [4], reservations exist concerning its nephrotoxicity and neurotoxicity, and these limit its use to a certain extent. However, reports of adverse reactions with CMS are inconclusive, and attempts to correlate toxicity with blood concentrations of CMS hinge upon an accurate assay method for CMS.

The established microbiological bioassay for colistin uses *Bordetella bronchiseptica* as the indicator organism and Difco no. 10 agar (BBL, Franklin Lakes, NJ, USA). This method is not used widely because of a lack of sensitivity, the inappropriateness of the indicator organism, and difficulties in obtaining this agar [5]. High-performance liquid chromatography assay methods for colistin have also been reported, but these require specialised equipment and technical expertise not present in many diagnostic laboratories [6]. In addition, there are issues concerning the correct analysis and quantification of colistin because of the complex mixture of derivatives obtained. In an attempt to address these problems, the present study reports the development of a bioassay that uses an unremarkable clinical isolate of *Escherichia coli* as the indicator organism.

The assay format used 100 mL of molten Diagnostic Sensitivity Test (DST) agar (Oxoid, Basingstoke, UK) supplemented with 130 mg of *p*-aminobenzoic acid (Sigma, Poole, UK). This was allowed to set in a sterile square 25 × 25 cm assay plate. A 10-mL inoculum containing 10^7 CFU/mL of the *E. coli* indicator strain was applied to the surface of the agar, which was then drained and allowed to dry for 30 min. Powdered CMS (Forest Laboratories, Bexley, UK) was dissolved in sterile distilled water to a concentration of 1 g/L, followed by dilution in pooled human serum to concentrations ranging from 0.5 to 128 mg/L. These standard concentrations were added, with a positive meniscus, to 10-mm wells cut in the agar. The samples were applied in

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Table 1. Accuracy and precision of colistin bioassay

Test	Target value (mg/L)	Mean value[a] (mg/L)	%CV
1	30	27.8	16.8
2	27	30.9	17.7
3	12	15.5	18.8
4	26	21.3	10.6
5	27	28.6	19
6	29	34.0	5.5
7	43	45.0	3.5
8	0.5	0.6	6.2
9	0.75	0.7	9
10	0.8	0.77	11.2
11	0.9	1.02	4

%CV, percentage coefficient of variation.
[a]Mean value obtained following triplicate determinations.

triplicate, after which the plates were pre-incubated at room temperature for 4 h, and then at 37°C for 24 h. The zones of inhibition were then measured and the mean zone diameters were plotted against log concentration to produce a standard curve [7].

The accuracy and precision of the method were assessed by assaying triplicate samples, spiked with known concentrations of CMS (Table 1), in comparison with the standard curve. Over the concentration range 0.5–43.0 mg/L, the mean accuracy was 93.7%, with an intra-assay precision of 3.5–18.8%. These figures are similar to those reported generally for other microbiological bioassays [8].

As CMS is known to undergo hydrolysis via a complex pathway that includes active products, the stability of serum samples spiked with known concentrations of CMS was examined. In samples stored at room temperature and at 4°C, CMS was stable for 7–10 days (< 10% change), after which levels decreased at room temperature (66% loss after 2 months), and increased slightly at 4°C (20% increase after 2 months). CMS was stable at −20°C and at −40°C for 21 days (< 10% change), after which antibacterial activity increased (33% increase after 2 months) as a result of hydrolysis into the more potent derivatives. At −70°C, CMS was stable for the duration of the 2-month test period. Therefore, it is advisable that samples for CMS assay should be stored at −70°C if the assay is to be delayed by more than a few days.

In conclusion, the microbiological bioassay for CMS described in this report is more accurate and sensitive than the method used previously, and has been used for the past 3 years for the assay of clinical samples. The bioassay can be used to assess CMS concentrations in urine and serum samples by comparison with standard concentrations of 8–128 mg/L and 0.5–8 mg/L, respectively. The bioassay is less complex than high-performance liquid chromatography methods, requires little specialised equipment, and is ideally suited for use in clinical microbiology laboratories.

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