ΕΞ. ΕΠΕΙΓΟΝ

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΥΠΟΥΡΓΕΙΟ ΥΓΕΙΑΣ
ΓΕΝΙΚΗ Δ/ΝΣΗ ΔΗΜΟΣΙΑΣ ΥΓΕΙΑΣ
& ΠΟΙΟΤΗΤΑΣ ΖΩΗΣ
Δ/ΝΣΗ ΔΗΜΟΣΙΑΣ ΥΓΕΙΑΣ
ΤΜΗΜΑ Α'
Πληροφορίες: Ο. Παντελά
Ταχ. Δ/νση: Αριστοτέλους 19
Ταχ. Κώδικας: 10187
Τηλέφωνο: 2132161325
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Αθήνα, 29 /05/2018
Αρ. Πρωτ. Δ1α/Γ.Π.οικ.40944

ΠΡΟΣ: Όπως Πίνακας Διανομής

ΘΕΜΑ: «Ελεγχος ευαισθησίας στην κολιστίνη σύμφωνα με τις διατάξεις της κοινής εργασίας CLSI/EUCAST για τα όρια ευαισθησίας στις πολυμιξίνες.».

ΣΧΕΤ.:

1.Το από 07-02-2018 έγγραφο της Εκτελεστικής Επιτροπής του ΚΕΣΥ με θέμα «Ελεγχος ευαισθησίας στην κολιστίνη (Πολυμιξίνη Ε) σύμφωνα με τις διατάξεις της κοινής εργασίας CLSI/EUCAST για τα όρια ευαισθησίας στις πολυμιξίνες» μετά των συνημμένων του.

2. Η από 01-08-2017 Απόφαση του Γεν. Γραμμ. Γ. Γιαννόπουλου (ΑΔΑ: ΩΠ89465ΦΥΟ-3ΕΠ «Σύσταση και ορισμός μελών της Εθνικής Επιτροπής Αντιβιογράμματος».

Σε συνέχεια του έργου της Εθνικής.Επιτροπής Αντιβιογράμματος, που συγκροτήθηκε με τη σχετ..(2) Απόφαση, αναφορικά με την παροχή τεχνικών συμβουλών για το αντικείμενο του αντιβιογράμματος στα κλινικά εργαστήρια, αποστέλλουμε το ανωτ. σχετ. (1) έγγραφο του ΚΕΣΥ και παρακαλούμε τους φορείς που εκτελούν αντιβιογράμματα για τη συμπλήρωση και αποστολή του ερωτηματολογίου που επισυνάπτεται στην ηλεκτρονική δ/νση pkommata@moh.go.gr.

ΣΥΝ: σελ. (10)

Η ΠΡΟΪΣΤΑΜΕΝΗ ΤΗΣ Δ/ΝΣΗΣ

ΕΣΩΤΕΡΙΚΗ ΔΙΑΝΟΜΗ

1. Γρ. Γενικών Γραμματέων
2. Γρ. Προϊστ. Γεν. Δ/νσης Υπηρεσιών Υγείας
3. Γρ. Προϊστ. Γεν. Δ/νσης Δημ. Υγείας & Π.Ζ.
4. Δ/νση Πρωτοβάθμιας Φροντίδας Υγείας
5. Δ/νση Οργάνωσης και Λειτουργίας Νοσ. Μονάδων & Εποπτευόμενων φορέων
6. Δ/νση Δημόσιας Υγείας

Β. ΚΑΡΑΟΥΛΗ

ΘΕΩΡΗΘΗΚΕ ΓΙΑ ΤΗΝ ΑΚΡΙΒΕΙΑ
Ο ΠΡΟΪΣΤΑΜΕΝΟΣ ΤΜΗΜΑΤΟΣ ΠΡΩΤΟΚΟΛΛΟΥ
& α.α.
ΑΝΝΑ-ΜΑΡΙΑ ΠΑΠΑΔΟΠΟΥΛΟΥ

ΠΙΝΑΚΑΣ ΔΙΑΝΟΜΗΣ

1. **Όλες τις Περιφερειακές Αυτοδιοικήσεις**
Υπόψη Περιφερειαρχών (Έδρες τους)
(Με την παράκληση να ενημερώσουν όλους τους φορείς ευθύνης τους)

2. **Όλες τις Υ.ΠΕ. (Έδρες τους)**
Υπόψη Διοικητών
(με την παράκληση να ενημερώσουν όλους τους εποπτευόμενους από αυτούς φορείς)

3. **Υπουργείο Εθνικής Άμυνας**
Δ/νση Υγειονομικού ΓΕΕΘΑ
(με την παράκληση να ενημερώσει όλους τους υγειονομικούς σχηματισμούς αρμοδιότητάς του)
Μεσογείων 227-231
Τ.Κ. 15451, Χολαργός

4. **Πανελλήνιος Ιατρικός Σύλλογος**
(με την παράκληση να ενημερωθούν όλοι οι Ιατρικοί Σύλλογοι της χώρας)
Πλουτάρχου 3, Τ.Κ.10675 - Αθήνα

5. **Υπουργείο Παιδείας Θρησκευμάτων Πολιτισμού & Αθλητισμού**
Ενιαίος Διοικητικός Τομέας Ανωτάτης Εκπαίδευσης
Γρ. Ειδικού Γραμματέα
(με την παράκληση να ενημερωθούν τα Νοσοκομεία ευθύν ους)
Ανδρέα Παπανδρέου 37 Μαρούσι ΤΚ 15180

ΚΟΙΝΟΠΟΙΗΣΗ:

1. **ΚΕΣΥ**
Μακεδονίας 8
Τ.Κ. 10433, Αθήνα

2. **ΚΕΕΛΠΝΟ (Γρ. Νοσ. Λοιμώξεων και Μικροβιακής Αντοχής)**
Υπόψη Προέδρου
Αγράφων 3-5
Τ.Κ. 15123, Μαρούσι

3. **ΕΚΕΠΥ**
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Μακεδονίας 8
Τ.Κ. 10433, Αθήνα

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΥΠΟΥΡΓΕΙΟ ΥΓΕΙΑΣ
ΚΕΝΤΡΙΚΟ ΣΥΜΒΟΥΛΙΟ ΥΓΕΙΑΣ
ΕΚΤΕΛΕΣΤΙΚΗ ΕΠΙΤΡΟΠΗ

Αθήνα, 7/2/ 2018

Προς: Δ/νση Οργάνωσης & Λειτουργίας
Νοσηλευτικών Μονάδων &
Εποπτευόμενων Φορέων

Θέμα: « Έλεγχος ευαισθησίας στην κολιστίνη (πολυμυξίνη Ε) σύμφωνα με τις συστάσεις της Κοινής Ομάδας Εργασίας CLSI/EUCAST για τα Όρια Ευαισθησίας στις Πολυμυξίνες»

Δεδομένης της έναρξης λειτουργ , της Εθνικής Επιτροπής Αντιβιογράμματος του ΚεΣΥ, σας αποστέλλουμε, συνημμένα στο παρόν, 1) έγγραφο του Προέδρου της Εθνικής Επιτροπής Αντιβιογράμματος, 2)σχετικό Ερωτηματολόγιο καθώς και 3)μελέτη που δημοσιεύτηκε στο CMI και αφορά στην colistin (9 σελίδες)
& παρακαλούμε για την διαβίβασή τους σε όλα τα Βιοπαθολογικά εργαστήρια των Νοσοκομείων του ΕΣΥ & ΠΑΝΕΠΙΣΤΗΜΙΑΚΑ της επικράτειας.
Επιπλέον, παρακαλούμε για την αντίστοιχη διαβίβασή τους στα Βιοπαθολογικά εργαστήρια των ΣΤΡΑΤΙΩΤΙΚΩΝ ΝΟΣΟΚΟΜΕΙΩΝ (αρμοδιότητας ΥΕΘΑ), στα ΝΟΣΟΚΟΜΕΙΑ «ΑΡΕΤΑΙΕΙΟ» & «ΑΙΓΙΝΗΤΕΙΟ» (αρμοδιότητας Υπ. Παιδείας) καθώς και στα Ιδιωτικά Βιοπαθολογικά Εργαστήρια της επικράτειας.
Επισημαίνονται τα ακόλουθα:
1)Η διαβίβαση στα Νοσοκομεία και στα Ιδιωτικά Εργαστήρια θα πρέπει να γίνει άμεσα & να φέρει τον χαρακτήρα του εξ. Επείγοντος.
2)Το συμπληρωμένο ερωτηματολόγιο να αποστέλλεται στην ηλ. δ/νση pkommata@moh.gov.gr
Προς διευκόλυνσή σας, τα συνημμένα έγγραφα σας αποστέλλεται & σε ηλεκτρονική μορφή στην ηλ. σας δ/νση damy@moh.gov.gr

Για την Εκτελεστική Επιτροπή του ΚεΣΥ
Ο Πρόεδρος

Καθηγητής Κώστας Β. Μάρκου

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΥΠΟΥΡΓΕΙΟ ΥΓΕΙΑΣ
ΚΕΝΤΡΙΚΟ ΣΥΜΒΟΥΛΙΟ ΥΓΕΙΑΣ
ΕΘΝΙΚΗ ΕΠΙΤΡΟΠΗ
ΑΝΤΙΒΙΟΓΡΑΜΜΑΤΟΣ

Αθήνα 29/1/2018

Προς : Εκτελεστική Επιτροπή
του ΚεΣΥ

ΘΕΜΑ: « Έλεγχος ευαισθησίας στην κολιστίνη (πολυμυξίνη Ε) σύμφωνα με τις συστάσεις της Κοινής Ομάδας Εργασίας CLSI/EUCAST για τα Όρια Ευαισθησίας στις Πολυμυξίνες»

Ο προσδιορισμός της ευαισθησίας στην κολιστίνη αποτελεί κρίσιμη παράμετρο στη θεραπεία των λοιμώξεων στην χώρα μας, τόσο λόγω του υψηλού επιπολασμού των πολυανθεκτικών βακτηριδίων όσο και της γνωστής τεχνικής δυσκολίας που παρουσιάζει με τη μέθοδο διάχυσης των δίσκων, τις ταινίες διαβαθμισμένης συγκέντρωσης και τα ημιαυτόματα συστήματα. Όσον αφορά τα τελευταία η EUCAST δεν τα αξιολόγησε συστηματικά, αλλά στέλνοντας στελέχη με τιμές MIC στο εύρος της μη ευαισθησίας σε συναδέλφους ανά τον κόσμο, διαπίστωσε τη συχνή εμφάνιση εξαιρετικά κρίσιμων σφαλμάτων (Very Major Errors). Γι' αυτό και συστήνει στους χρήστες ημι-αυτόματων συστημάτων να διενεργούν αυστηρούς εσωτερικούς ελέγχους ποιότητας και να ελέγχουν με τους αντίστοιχους κατασκευαστικούς οίκους κατά πόσο μπορούν να είναι σίγουροι ότι η ημι-αυτόματη μέθοδος ελέγχου ευαισθησίας δίνει σωστά αποτελέσματα για την κολιστίνη.

Λόγω αυτών των τεχνικών δυσκολιών το CLSI και το EUCAST δημιούργησαν κοινή ομάδα εργασίας για τα όρια ευαισθησίας στις πολυμυξίνες (joint CLSI-EUCAST polymyxin breakpoints working group), που κατέληξε σε σχετικές οδηγίες /συστάσεις για τον έλεγχο ευαισθησίας κλινικών στελεχών Enterobacteriaceae, *P.aeruginosa* και *Acinetobacter* spp. στην κολιστίνη, οι οποίες είναι ανηρτημένες στην ιστοσελίδα www.eucast.org από τις 22 Μαρτίου 2016 και οι οποίες πρέπει να ακολουθούνται από όλα τα κλινικά εργαστήρια. Τις συστάσεις αυτές σας τις στέλνουμε σε μετάφραση.

Επισημαίνουμε ότι ο εσωτερικός έλεγχος ποιότητας για την κολιστίνη πρέπει να διενεργείται τακτικά, χρησιμοποιώντας ένα ευαίσθητο πρότυπο στέλεχος (*E. coli* ATCC 25922 με εύρος MICs 0.25-2.0 mg/L και στόχο 0.5-1 mg/L ή *P. aeruginosa* ATCC 27853 με εύρος MICs 0.5-4.0 mg/L και στόχο 1-2 mg/L) και το κολιστίνη ανθεκτικό *E. coli* NCTC 13846 (*mcr*-1 θετικό). Για το τελευταίο στέλεχος, η τιμή στόχος για την MIC στην κολιστίνη είναι 4 mg/L και μόνο περιστασιακά μπορεί να είναι 2 ή 8 mg/L (± 1 αραίωση σε σχέση με την MIC στόχο). Μέχρι τα εργαστήρια να προμηθευτούν το NCTC 13846, μπορούν να χρησιμοποιούν το *E. coli* 4320 που εστάλη από το EARS-Net (Σεπτ 2017) και έχει MIC 4mg/L.

Τέλος, σας ενημερώνουμε ότι προς το παρόν στην ελληνική αγορά κυκλοφορούν 2 προϊόντα για τον έλεγχο της MIC στην κολιστίνη με τη μέθοδο των μικροαραιώσεων σε ζωμό, το MICRONAUT MIC-Strip (Merlin Diagnostika) και το SensiTest (Liofilchem).

Πληροφορίες

- Ευαγγελία Λεμπέση, τηλ 2132009324 /2132009266

- Ευσταθία Περιβολιώτη, τηλ 2132043202
- Κυριακή Τρυφινοπούλου, τηλ 2108921077, 78

Συστάσεις κοινής ομάδας εργασίας CLSI-EUCAST για τα όρια ευαισθησίας στις πολυμυξίνες

Ο προσδιορισμός της MIC στην κολιστίνη (πολυμυξίνη Ε) σχετίζεται με διάφορα μεθοδολογικά θέματα. Η κοινή ομάδα εργασίας CLSI-EUCAST μελέτησε εκτενώς τα θέματα αυτά και συμφώνησε τα εξής:

1. Η μέθοδος αναφοράς για Enterobacteriaceae, *Pseudomonas aeruginosa* και *Acinetobacter* spp είναι η μέθοδος μικροαραιώσεων σε ζωμό, προτυποποιημένη κατά ISO (20776-1). Σημειώστε:
   α. Χρησιμοποιείται ζωμός Mueller-Hinton cation-adjusted

   β. Δεν πρέπει να προστίθεται καμία ουσία σε κανένα βήμα της διαδικασίας (ιδιαίτερα polysorbate-80 ή άλλες επιφανειοδραστικές ουσίες)

   γ. Οι πλάκες θα πρέπει να είναι κατασκευασμένες από καθαρό πολυστυρένιο και να μην προηγείται της χρήσης τους καμία περαιτέρω επεξεργασία

   δ. Πρέπει να χρησιμοποιούνται θειϊκά άλατα πολυμυξινών (το μεθανοσουλφονικό παράγωγο της κολιστίνης δεν πρέπει να χρησιμοποιείται καθώς είναι ένα ανενεργό προ-φάρμακο το οποίο διασπάται αργά σε διάλυμα)

2. Ο έλεγχος ευαισθησίας με άλλες μεθόδους, όπως η μέθοδος αραιώσεων σε αγαρ, η μέθοδος διάχυσης των δίσκων και η χρήση ταινιών διαβαθμισμένης συγκέντρωσης του αντιβιοτικού δεν μπορεί να προταθεί, μέχρις ότου ανασκοπηθούν όλα τα ιστορικά δεδομένα ή προκύψουν δεδομένα από καινούριες μελέτες. Οι μελέτες επί των μεθόδων αυτών βρίσκονται σε εξέλιξη.

Για την Εθνική επιτροπή Αντιβιογράμματος

Ο Πρόεδρος

Καθηγητής ΔΑΪΚΟΣ ΓΕΩΡΓΙΟΣ

ΕΘΝΙΚΗ ΕΠΙΤΡΟΠΗ ΑΝΤΙΒΙΟΓΡΑΜΜΑΤΟΣ

ΕΡΩΤΗΜΑΤΟΛΟΓΙΟ ΕΡΓΑΣΤΗΡΙΩΝ ΒΙΟΠΑΘΟΛΟΓΙΑΣ / ΜΙΚΡΟΒΙΟΛΟΓΙΚΟΥ ΤΜΗΜΑΤΟΣ

ΠΡΟΣΑΡΜΟΓΗ ΣΤΙΣ ΟΔΗΓΙΕΣ EUCAST

Α. ΣΤΟΙΧΕΙΑ ΕΡΓΑΣΤΗΡΙΟΥ		
ΝΟΣΟΚΟΜΕΙΟ		
ΣΤΟΙΧΕΙΑ ΔΙΕΥΘΥΝΤΟΥ ή ΥΠΕΥΘΥΝΟΥ ΕΡΓΑΣΤΗΡΙΟΥ		
ΟΝΟΜΑ:		
ΤΗΛΕΦΩΝΟ:		
E-MAIL:		
Β. ΕΛΕΓΧΟΣ ΕΥΑΙΣΘΗΣΙΑΣ ΣΤΑ ΑΝΤΙΜΙΚΡΟΒΙΑΚΑ		
		Παρακαλώ σημειώστε με Χ ότι εφαρμόζετε στο εργαστήριο
ΜΕΘΟΔΟΣ ΔΙΣΚΩΝ/ΔΙΑΧΥΣΗΣ ΣΕ ΑΓΑΡ	ΝΑΙ	
	ΟΧΙ	
ΠΡΟΣΔΙΟΡΙΣΜΟΣ MIC	ΝΑΙ	
	ΟΧΙ	
ΤΑΙΝΙΕΣ ΔΙΑΒΑΘΜΙΣΜΕΝΗΣ ΣΥΓΚΕΝΤΡΩΣΗΣ	ΝΑΙ	
	ΟΧΙ	
ΜΕΘΟΔΟΣ ΑΡΑΙΩΣΕΩΝ ΣΕ ΖΩΜΟ	ΝΑΙ	
	ΟΧΙ	
ΜΕΘΟΔΟΣ ΑΡΑΙΩΣΕΩΝ ΣΕ ΑΓΑΡ	ΝΑΙ	
	ΟΧΙ	
ΧΡΗΣΗ ΕΤΟΙΜΩΝ ΘΡΕΠΤΙΚΩΝ ΥΛΙΚΩΝ	ΝΑΙ	
	ΟΧΙ	
ΧΡΗΣΗ IN HOUSE ΘΡΕΠΤΙΚΩΝ ΥΛΙΚΩΝ	ΝΑΙ	
	ΟΧΙ	
ΑΥΤΟΜΑΤΟ ΣΥΣΤΗΜΑ	ΝΑΙ	
	ΟΧΙ	
ΟΔΗΓΙΕΣ ΠΟΥ ΕΦΑΡΜΟΖΕΤΕ		
CLSI	ΝΑΙ	
	ΟΧΙ	
EUCAST	ΝΑΙ	
	ΟΧΙ	

Παρακαλώ συμπληρώστε: (Α) τα στοιχεία του εργαστηρίου και
(Β) τις μεθόδους που χρησιμοποιείτε για τον έλεγχο της ευαισθησίας στα αντιμικροβιακά και αποστείλατε το ερωτηματολόγιο με EMAIL: pkommata@moh.gov.gr

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Original article

Antimicrobial susceptibility testing of colistin – evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp.

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ABSTRACT

Objective: Both EUCAST and CLSI recommend broth microdilution (BMD) for antimicrobial susceptibility testing of colistin, but BMD is rarely used in routine microbiology laboratories. The objective of this study was to evaluate five commercially available BMD products and two of the commercially available kinds of gradient tests for colistin MIC determination using BMD according to ISO standard 20776-1 as reference.

Methods: Colistin MIC determination was performed according to the manufacturer's instructions on five commercially available BMD products (Sensititre, MICRONAUT-S, MICRONAUT MIC-Strip, SensiTest, and UMIC) and two gradient tests (Etest and MIC Test Strip). Colistin reference MICs were determined using frozen panels according to ISO standard 20776-1. An international collection of Gram-negative bacteria ($n=75$) with varying levels of colistin susceptibility was tested.

Results: The colistin BMD products correlated well with reference MICs, in particular for Sensititre and the two MICRONAUT products (essential agreement ≥96%: 66/69 (96%, CI 88–99%), 72/75 (96%, CI 88–99%) and 74/75 (99%, CI 92–100%)). The results were somewhat poorer for the BMD products SensiTest and UMIC: EA 88% (51/58, CI 77–95%) and 82% (61/74, CI 72–89%), respectively), and considerably poorer for the gradient tests (EA 43–71% depending on gradient test and Mueller-Hinton agar manufacturer). The gradient tests generally underestimated colistin MICs, resulting in a significant number of false susceptible results (9–18 of total 75 tests, compared with 1–3 for the BMD products).

Conclusions: Based on the results of this study, we advise laboratories not to trust gradient tests for colistin susceptibility testing and to use broth microdilution methods for this purpose. There are several commercial broth microdilution tests available and in principle they perform well. E. **Matuschek, Clin Microbiol Infect 2017;■:1**

Introduction

An accurate method for antimicrobial susceptibility testing (AST) of colistin (polymyxin E) is crucial in an era of increasing numbers of multi-resistant Gram-negative bacteria and simultaneous increasing colistin resistance. The reference methodology for AST is MIC determination with broth microdilution (BMD) according to the ISO standard 20776-1 [1]. However, BMD of colistin is associated with methodological issues. Colistin binds to the plastic of polystyrene trays and attempts have been made to prevent this by adding a surfactant, such as polysorbate-80, to the test system [2,3]. Recently, a joint CLSI-EUCAST working group investigated colistin BMD testing and decided that the recommendations in the ISO standard should be adhered to and that testing should be performed using the sulphate salt of colistin and standard polystyrene trays without the addition of surfactants [4]. The working group showed that surfactants did not improve assay performance and that there is, in fact, a synergistic effect with colistin (J. Turnidge, personal communication).

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1198-743X/

Clinical microbiology laboratories only rarely perform reference broth microdilution, which requires freshly prepared or frozen antibiotic solutions. However, a number of more user-friendly commercial products for colistin BMD have recently become available. Methods widely used for AST at clinical laboratories are gradient tests, disk diffusion, and semi-automated devices. For many years, both CLSI and EUCAST have advised against the use of disk diffusion testing for colistin AST. Gradient tests and semi-automated AST devices have been extensively used at clinical laboratories, despite the problems reported with colistin AST on these systems [5,6].

The objective of this study was to evaluate five commercially available BMD products and the two available gradient tests for colistin MIC determination using frozen BMD panel MICs as reference. It was beyond the scope of the present investigation to evaluate semi-automated AST devices.

Materials and methods

Antimicrobial susceptibility testing was performed on an international collection of Gram-negative bacteria (n=75): *Escherichia coli* (n=14), *Klebsiella pneumoniae* (n=18), *Pseudomonas aeruginosa* (n=21), and *Acinetobacter* spp. (n=22, of which 16 were *Acinetobacter baumanii*) with varying levels of colistin susceptibility, kindly provided by Paul Rhomberg, JMI Laboratories, USA (isolates from the worldwide SENTRY surveillance program); Sören Gatermann, Bochum, Germany; Rene Henriksen, Copenhagen, Denmark; Ørjan Samuelsen, Tromsø, Norway; Jordi Vila, Barcelona, Spain; and Luis Martinez-Martinez, Santander, Spain.

The isolates were identified to species level using the Microflex system with the MALDI Biotyper 3.1 software (Bruker Daltonics) and the MBT database-5627 according to the manufacturer's instructions. Colistin reference MICs were determined in accordance with the ISO standard 20776-1 [1] and CLSI/EUCAST recommendations [4] on frozen BMD panels (Thermo Fisher Scientific, Cleveland, OH, USA) with two-fold dilutions from 128 to 0.125 mg/L. MIC determination for colistin was performed according to the manufacturers' instructions for seven commercially available MIC products. Five were BMD products with freeze-dried antibiotics: SEMPA1 (custom Sensititre plate, Thermo Fisher Scientific, East Grinstead, UK), MICRONAUT-S and MICRONAUT MIC-Strip (MERLIN Diagnostika Gmbh, Bornheim, Germany), SensiTest (Liofilchem, Roseto degli Abruzzi, Italy) and UMIC (Biocentric, Bandol, France). The Sensititre plate is designed to test one isolate against several antimicrobial agents including colistin, whereas the other products are for testing colistin only. The MICRONAUT-S is a 96-well panel for eight isolates, the SensiTest consists of a smaller panel for four isolates and the MICRONAUT MIC-Strip and UMIC are single-isolate tests consisting of a plastic device with 12 wells. Skipped wells were observed when reading the BMD panels, the isolates were retested. The two gradient test brands available at the time of the study, Etest (bioMérieux, Marcy l'Etoile, France) and MIC Test Strip (MTS, Liofilchem), were also investigated. Etest and MTS were tested on in-house prepared Mueller-Hinton (MH) agar plates using agar powder from Oxoid (Thermo Fisher Scientific, Basingstoke, UK) and BBL (BD, Sparks MD, USA) in parallel. Etest was also tested on the bioMérieux's Mueller Hinton E (MHE) medium as recommended by the manufacturer. The fully colistin-susceptible *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 and the *mcr1*-positive *E. coli* NCTC 13846 (CCUG 70662, DSM 105182) with a colistin MIC of 4 mg/L were used as quality control (QC) for all methods (≥6 tests per strain and method) and analysed vs. EUCAST QC Tables version 7.0 [7]. Essential agreement (EA = MICs within ± 1 dilution of reference MICs) and categorical agreement (CA) were calculated according to ISO standard 20776-2 [8] vs. EUCAST Breakpoint Tables version 7.1 [9] using colistin MICs on frozen BMD panels as reference (susceptible ≤2, resistant >2 mg/L). There are no CLSI breakpoints for Enterobacteriaceae, but CLSI breakpoints for *P. aeruginosa* and *Acinetobacter* spp. are the same as for EUCAST [10]. For the BMD products Sensititre, SensiTest, and UMIC, the number of tests used to calculate the EA was lower than the total number of isolates because of truncations in the MIC panel ranges. The numbers of isolates included per the total numbers of isolates for Enterobacteriaceae (*E. coli* and *K. pneumoniae*), *P. aeruginosa* and *Acinetobacter* spp., respectively, were: Sensititre (28/32, 19/21, 22/22) SensiTest (26/32, 15/21, 17/22), and UMIC (32/32, 20/21, 22/22). The MIC values of the isolates not included in the EA calculations were either ≤0.25 or ≥32 mg/L (Fig. 1).

The occurrence of *mcr* genes was investigated by whole-genome sequencing (WGS) all isolates with colistin reference MICs 2–8 mg/L (n=24) and the three Enterobacteriaceae with reference MICs 1 mg/L.

Results

Colistin reference MICs for the 75 Gram-negative bacteria were from 0.25 to 128 mg/L (Table 1). A total of 24 isolates had MICs of 2, 4, and 8 mg/L, that is just below, on, and above the EUCAST breakpoints (susceptible ≤2, resistant >2 mg/L). The correlation with reference MICs was good for all BMD products with an expected 45-degree correlation (1:1 correspondence in the linear regression) across the full scale of MIC values (Fig. 1). However, skipped wells which required retesting occurred occasionally on all BMD panels. The correlation with reference MICs was poor for gradient tests and a 45-degree correlation could not be obtained with either of the gradient test-medium combinations tested (Fig. 1). The correlation for the gradient tests was especially poor for isolates with MICs above the breakpoint (>2 mg/L).

None of the *P. aeruginosa* or *Acinetobacter* spp. analysed with WGS (colistin MICs 2–8 mg/L) contained any *mcr* genes. All *E. coli* with colistin 4 mg/L were positive for *mcr-1*, as well as one *K. pneumoniae* with 8 mg/L. One colistin-resistant *E. coli* (8 mg/L) contained both *mcr-1* and *mcr-3*. One colistin-susceptible *E. coli* (1 mg/L) was positive for *mcr-1* but tested susceptible with all methods.

Essential agreement

The highest essential agreement (EA 96–99%) was obtained for Sensititre and the two MICRONAUT products (essential agreement ≥96%: 66/69 (96%, CI 88–99%), 72/75 (96%, CI 88–99%), and 74/75 (99%, CI 92–100%)), see Table 2. For the broth microdilution single-isolate test from MICRONAUT (MIC-Strip), only one MIC was outside essential agreement. The results were poorer for SensiTest and UMIC, with EA of 88% (51/58, CI 77–95%) and 82% (61/74, CI 72–89%), respectively. The lowest EA was obtained for the gradient tests, which varied between 43% (32/75, CI 32–54%) and 71% (53/75, CI 60–80%), depending on the MH medium used (Table 2). The correlation with reference MICs for gradient tests was best for Etest on Oxoid MH and poorest for Etest on BBL MH agar.

For Sensititre and the two MICRONAUT products, the tests performed well for all species investigated (EA 91–100% depending on species). For the two other BMD products, the test performance varied depending on the species investigated, with poorer performance (EA <80%) for *Acinetobacter* spp. on SensiTest and for both *P. aeruginosa* and *Acinetobacter* spp. on UMIC. For the gradient tests, there were marked differences between the species investigated, with the poorest EA for *Acinetobacter* spp. which was below 10% (1/22 (5%, CI 1–22%) and 2/22 (9%, CI 3–28%) for Etest on BBL MH and MHE. However, also for *E. coli*, *K. pneumonia*, and *P. aeruginosa*, EA was generally low.

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Categorical agreement

The categorical agreement (number of tests with correct susceptibility categorization) varied from 89% to 95% for the BMD products and from 76% to 85% for the gradient tests. The BMD products tended to overestimate MICs to a small extent, both for susceptible and resistant isolates (Fig. 1), resulting in some major errors, that is false resistant results (Table 2). Most of these (12/25) were for *Acinetobacter* spp. There were also a few very major errors (false susceptible results) for the two MICRONAUT tests, SensiTest and UMIC, and the majority of these (6/8) were for *P. aeruginosa*. Gradient tests generally underestimated MICs, especially in the area above the breakpoint, resulting in a significant number of very major errors, that is false susceptible results (9–18 per test-medium combination of a total of 75 tests), whereas false resistant results were few (0–2).

Most QC results were within acceptable ranges (Table 3), but readings below the range for *E. coli* ATCC 25922 were observed for MICRONAUT MIC-Strip (1/8) and UMIC (3/8). For Etest, all MICs were out of range for *E. coli* ATCC 25922 on BBL and MHE agar, whereas most MICs were within range for *P. aeruginosa* ATCC 27853 (12/12 on BBL MH and 4/7 on MHE). For MTS, all MICs were within range for the two susceptible QC strains, and 13/14 of these MICs were on the target values. For the *mcr-1* positive *E. coli* NCTC 13846, MICs for both BMD and gradient tests ranged from 2 to 8 mg/L, with 42/48 BMD results and 35/40 gradient test results at the expected 4 mg/L.

Discussion

Colistin is normally the last resort agent used in the treatment of serious infections caused by multi-resistant Enterobacteriaceae, *Pseudomonas aeruginosa*, or *Acinetobacter* spp. A false susceptible result is obviously a very major error (VME) but in a last resort agent, a false resistant result is just as unfortunate and should be considered equally serious. It means that with colistin, it is

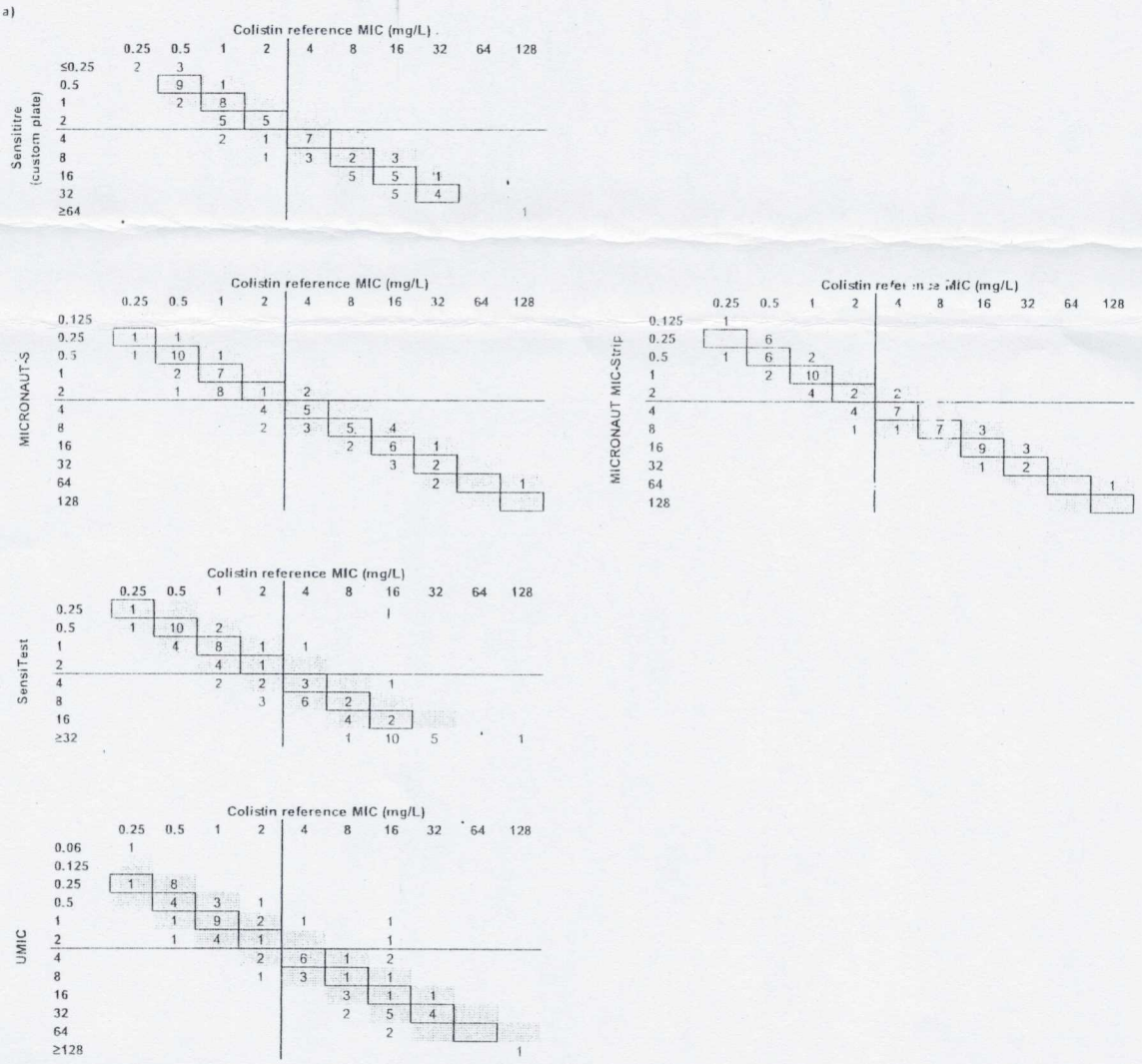

Fig. 1. Correlation between test methods and reference broth microdilution for (a) five BMD products (Sensititre custom plate, MICRONAUT-S, MICRONAUT MIC-Strip, SensiTest and UMIC) and (b) two gradient tests (Etest and MTS) for 75 Gram-negative bacterial isolates. For gradient tests, results are shown per Mueller–Hinton (MH) agar. MICs within essential agreement (within ± 1 dilution of reference MICs) are highlighted in grey and MICs identical with reference MICs are within boxes. EUCAST breakpoints (susceptible ≤2, resistant >2 mg/L) are shown as lines.

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b)

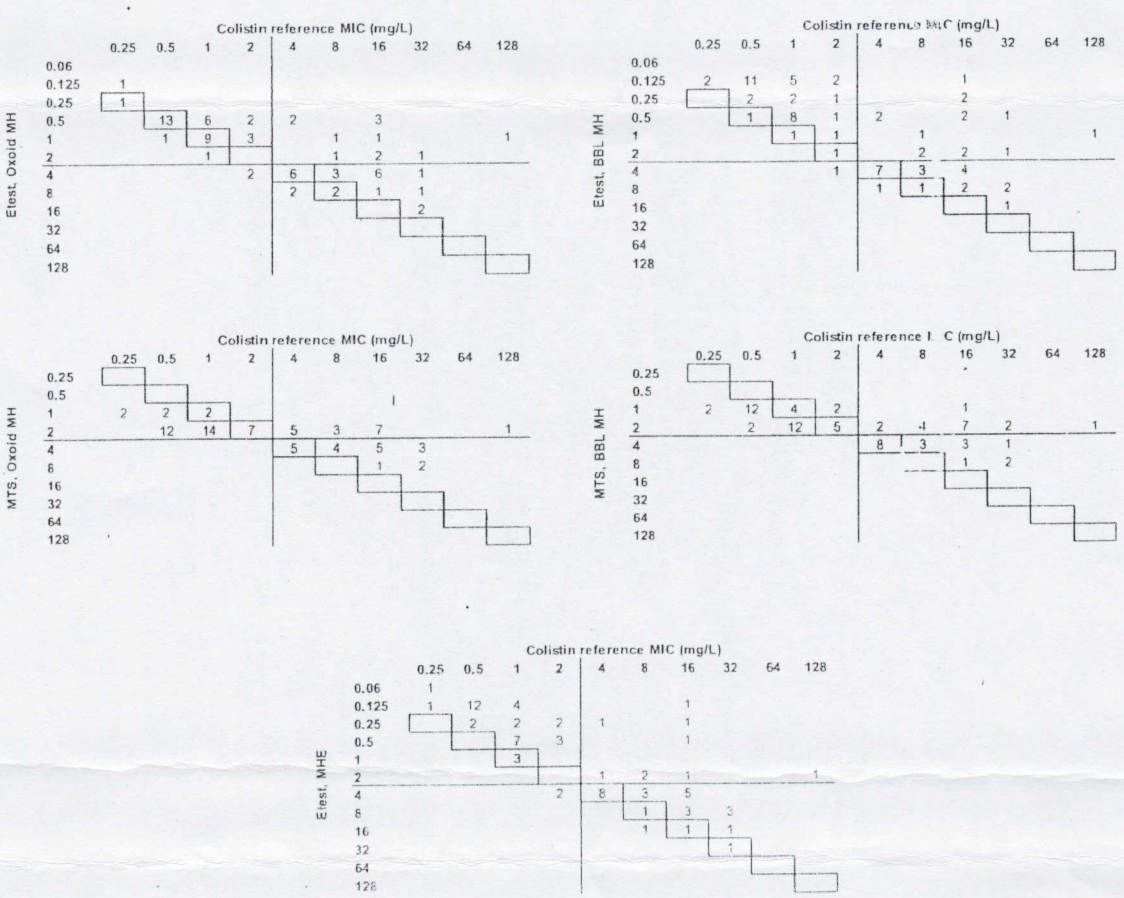

Fig. 1. (*continued*).

Table 1
Colistin MIC distributions with reference broth microdilution for 75 Gram-negative bacterial isolates

Organism	Number of isolates	Colistin reference MIC (mg/L)									
		0.25	0.5	1	2	4	8	16	32	64	128
Escherichia coli	14	1	3	1		8	1				
Klebsiella pneumoniae	18		4	2	2		4	4	2		
Pseudomonas aeruginosa	21	1	2	7	2	2	2	[illegible]	1		1
Acinetobacter spp.	22	[illegible]	5	6	3			6	2		
Total	75	2	14	16	7	10	7	11	5	0	1

absolutely essential for laboratories to report correct results and good essential agreement is more important than for many other antimicrobial agents.

Gradient tests performed slightly better for isolates that lacked colistin resistance mechanisms (MICs ≤2 mg/L) than for colistin-resistant isolates (MICs >2 mg/L). For some time, it was hoped that a susceptible result could be trusted even if the ability to predict the level of resistance was poor. However, our results indicate that although isolates without colistin resistance were mostly categorized as susceptible, isolates with colistin resistance mechanisms could be categorized as susceptible or resistant. Furthermore, the essential agreement was poor also for susceptible isolates, resulting in underestimation of colistin MICs for both susceptible and resistant isolates. It is likely that the poor correlation between gradient tests and BMD reference MICs is related to the poor diffusion of colistin in agar. A similar poor correlation was observed for disk diffusion, which was performed in parallel using colistin disks with three potencies (10, 25, and 50 μg) on a subset of the isolates in this study (data not shown). None of the disks could discriminate between colistin susceptible and resistant isolates. The gradient tests performed better for *E. coli* and *K. pneumoniae* than for *P. aeruginosa* and *Acinetobacter* spp., but in our opinion, the major problems with colistin gradient tests shown in this study deem those products unreliable for colistin MIC determination in any species.

Commercial BMD products correlated significantly better with reference methodology than the gradient tests. False susceptible results (very major errors) were mainly obtained for *P. aeruginosa*, which is not surprising as the susceptible breakpoint was set at ≤2 mg/L, whereas the epidemiological cut-off (ECOFF) is 4 mg/L.

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Table 2
Essential and categorical agreements for colistin MIC tests for 75 Gram-negative bacteria with MICs on frozen broth microdilution panels as reference

	Organism	*E. coli* and *K. pneumoniae* (*n*=32)	*P. aeruginosa* (*n*=21)	*Acinetobacter* spp. (*n*=22)	All isolates (*n*=75)
	Colistin reference MIC range (mg/L)	0.25–32	0.25–128	0.5–32	0.25–128
% Essential agreement (EA)[a]	Sensititre custom plate[b]	96	100	91	96
	MICRONAUT-S	97	100	91	96
	MICRONAUT MIC-Strip	97	100	100	99
	SensiTest[c]	96	93	71	88
	UMIC[d]	91	75	77	82
	Etest, Oxoid MH	84	62	59	71
	Etest, BBL MH	63	52	45	43
	Etest, MHE	75	43	91	47
	MTS, Oxoid MH	59	57	41	53
	MTS, BBL MH	75	57	59	65
% Categorical agreement (CA)[e]	Sensititre custom plate	97	95	91	95
	MICRONAUT-S	94	86	86	89
	MICRONAUT MIC-Strip	94	91	86	91
	SensiTest	94	91	82	89
	UMIC	94	91	91	92
	Etest, Oxoid MH	94	71	73	81
	Etest, BBL MH	94	67	68	79
	Etest, MHE	94	76	82	85
	MTS, Oxoid MH	81	71	82	79
	MTS, BBL MH	84	71	68	76
Number of major errors (ME)[f]	Sensititre custom plate	1	1	2	4
	MICRONAUT-S	2	1	3	6
	MICRONAUT MIC-Strip	2	0	3	5
	SensiTest	2	1	4	7
	UMIC	2	1	0	3
	Etest, Oxoid MH	2	0	0	2
	Etest, BBL MH	1	0	0	1
	Etest, MHE	2	0	0	2
	MTS, Oxoid MH	0	0	0	0
	MTS, BBL MH	0	0	0	0
Number of very major errors (VME)[g]	Sensititre custom plate	0	0	0	0
	MICRONAUT-S	0	2	0	2
	MICRONAUT MIC-Strip	0	2	0	2
	SensiTest	0	1	0	1
	UMIC	0	1	2	3
	Etest, Oxoid MH	0	6	6	12
	Etest, BBL MH	1	7	7	15
	Etest, MHE	0	5	4	9
	MTS, Oxoid MH	6	6	4	16
	MTS, BBL MH	5	6	7	18

[a] MICs being within ± 1 dilution of reference MICs.
[b] Because of truncations in the MIC dilutions, the total number of tests for calculation of EA was 28 for *E. coli*/*K. pneumoniae* and 19 for *P. aeruginosa*.
[c] Because of truncations in the MIC dilutions, the total number of tests for calculation of EA was 26 for *E. coli*/*K. pneumoniae*, 15 for *P. aeruginosa* and 17 for *Acinetobacter* spp.
[d] Because of truncations in the MIC dilutions, the total number of tests for calculation of EA was 20 for *P. aeruginosa*.
[e] Test results with correct susceptibility categorization.
[f] Resistant with test method, susceptible with reference method = false resistant.
[g] Susceptible with test method, resistant with reference method = false susceptible.

It is therefore likely that even a well-calibrated method will, to some extent, underestimate colistin resistance in *P. aeruginosa*.

As discussed by several other authors, colistin MIC determination is associated with methodological difficulti [2,3,11,12]. This study was not designed to further investigate t fect of adding surfactants or other modifications to the refer methodology, but to evaluate commercial products for colistin MIC determination. Our results show that colistin MIC determination can be performed with reproducible results using both reference BMD methodology and commercial BMD products.

When analysing the quality control (QC) data, it was obvious that the regular susceptible QC strains could not disclose the poor ability of gradient tests to predict colistin resistance. Furthermore, the QC ranges, consisting of four two-fold dilutions, recommended by both EUCAST and CLSI for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 28753 allow significant variation in colistin MICs regardless of method used. Such variation is not acceptable when testing clinical isolates. We strongly recommend that the colistin-resistant *E. coli* NCTC 13846 (CCUG 70662, DSM 105182) is included in all colistin susceptibility testing. For this strai , the expected colistin MIC is 4 mg/L, and in our experience it is reasonable to expect almost all results between 2 and 8 mg/L and ≥ 80% on 4 mg/L.

BMD is commonly regarded as a laborious and expensive method, but the commercial BMD products evaluated in this study are easy to use and do not require additional equipment or great expertise. It should therefore be possible for clinical microbiology laboratories to internally validate any of these products for reliable colistin MIC testing and to completely stop using gradient tests for this purpose.

All testing in this study was performed by skilled staff in a laboratory performing broth microdilution daily and quality control was performed throughout the study. Ideally, we would have performed all tests on the same day, from the same inoculum suspension, with the same Mueller-Hinton broth, etc., but this was not logistically possible, which is a limitation of this study. We believe the comparisons were as fair as is possible. We would, however, like to stress that when evaluating antimicrobial susceptibility tests, it is possible to achieve results which are better or worse by choosing

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Table 3
Colistin quality control results per MIC method

Colistin MIC method	Colistin MIC (mg/L)											
	Escherichia coli ATCC 25922					*Pseudomonas aeruginosa* ATCC 27853				*Escherichia coli* NCTC 13846[a]		
	0.125	0.25	0.5	1	2	0.25	0.5	1	2	4	2	4	8
Broth microdilution													
Reference frozen panel			7	1			8				1	7	
Sensititre custom plate		4	4				1	7				8	
MICRONAUT-S		5	3					4	4			7	1
MICRONAUT MIC-Strip	1	6	1					8			2	6	
SensiTest			5	1				7				7	
UMIC	3	3	2					5	1		2	7	
Gradient tests													
Etest, Oxoid MH		2	5					7				8	
Etest, BBL MH	12						4	8				8	
Etest, MHE	7					3	4				*5	3	
MTS, Oxoid MH				6	1			2	5			8	
MTS, BBL MH			1	6				4	3			8	

Acceptable ranges are highlighted in grey and results on target v[illegible] are bold.
[a] *mcr-1* positive.
[b] All four values at ≤0.25 mg/L.

easier or more difficult isolates for the evaluation. If isolates are chosen which are clearly at different ends of the spectrum of susceptibility, numbers of errors are going to be low. If isolates close to the breakpoints are chosen, numbers of errors are going to be higher. On the other hand, if breakpoints allow for a wide intermediate category, there will be very few major or very major errors. In this study, isolates were difficult and many of them had colistin MIC values close to the breakpoints. As neither EUCAST nor CLSI have introduced an intermediate category, errors will be either major errors or very major errors. These factors make our comparison a challenging one to the tests we evaluated.

Conclusions

Commercial broth microdilution methods generally performed well with the best correlation for Sensititre and the two MICRONAUT tests, whereas the performance of the two gradient tests was unacceptable. This is probably related to the poor, and possibly unpredictable, diffusion of colistin in agar.

Based on the results of this study, we advise laboratories not to trust colistin gradient tests or disk diffusion and to use broth microdilution methods for this purpose. This advice has been adopted by EUCAST. There are several commercial and user-friendly broth microdilution tests available on the market. However, a favourable result for a commercial product in this study does not mean that EUCAST recommends or endorses this particular product. The need for stringent quality control of any method is emphasized and we recommend that all laboratories performing colistin MIC determination include the colistin resistant *E. coli* NCTC 13846 for quality control. The colistin MIC target value for this strain is 4 mg/L and should only occasionally be 2 or 8 mg/L. We did not have the opportunity to validate the performance of semi-automated AST devices in this study, but others have reported poor performance for colistin with these [5,6].

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Transparency declaration

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