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Clinical Validation of SensiTest Colistin, a Broth Microdilution-Based Method To Evaluate Colistin MICs

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ABSTRACT The global spread of multidrug-resistant Gram-negative bacteria has led to the return of colistin for treating severe infections. Recently, different plasmid-mediated genes conferring resistance to this drug were described and reported worldwide. International committees (EUCAST/CLSI) reevaluated inconsistencies surrounding colistin antimicrobial susceptibility testing (AST), concluding that broth microdilution (BMD) should serve as the reference method for AST. The development of an accurate, reproducible commercial test based on BMD is therefore highly desirable. SensiTest Colistin (STC), a BMD-based compact 4-test panel containing the lyophilized antibiotic in 7 2-fold dilutions (0.25 to 16 μg/ml) was here compared with the EUCAST-CLSI standard reference method (BMD) and, for some isolates, with the automated Phoenix 100 system (PHX). A total of 353 bacterial strains were evaluated by two different laboratories; 137 isolates were resistant to colistin (19 were intrinsically resistant, 83 harbored the *mcr-1* gene). Essential agreement (EA) between STC and BMD was obtained for 339 out of the 353 strains tested (96.0%). Overall categorical agreement was obtained for 349 out of the 353 strains analyzed (98.9%). Two major errors (MEs; 0.93%) and two very major errors (VMEs; 1.46%) were documented. STC appeared to be a simple but highly reliable test with good reproducibility even with panels stored at room temperature or at 35°C. Moreover, STC showed a good performance with strains carrying the *mcr-1* gene, with a 98.8% EA. As the secondary endpoint of our study, VMEs for PHX were documented for 6 isolates (10%).

KEYWORDS multidrug resistance, colistin, MIC, SensiTest Colistin, antimicrobial susceptibility testing, Phoenix 100 system, Liofilchem, Becton Dickinson

The antibiotic properties of polymyxins, originally derived from strains of *Paenibacillus* (*Bacillus*) *polymyxa*, were first described in the 1940s. Formerly studied in the 1950s (1) and used for some years in the treatment of Gram-negative bacterial infections, colistin (polymyxin E) is the drug most widely used in clinical practice. However, with growing concern over significant side effects, colistin lost importance in comparison to emerging more efficacious drugs with less overt toxicity (2) and was removed from use some decades later.

The global spread of multidrug-resistant Gram-negative bacteria (MDRGNB) has led to a distinct limitation in the therapeutic options available. This has seen the return of colistin to the clinical arena (3), albeit it has been reassessed to better define its dosage and daily administration (4). Colistin has often become the last option to treat severe infections caused by MDRGNB, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and carbapenem-resistant *Enterobacteriaceae* (CRE), being frequently used in a dual-therapy regimen.

Until recently, colistin resistance was always thought to be chromosomally encoded

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and mutationally acquired, allowing vertical transmission only, and, thus, by its very nature, rare and self-limiting. This kind of colistin resistance is determined by mutations in a wide variety of species-specific mechanisms: *mgrB* and *ccrB* in *Klebsiella pneumoniae* and *parS* and *cprS* in *P. aeruginosa*, in addition to the more widely dispersed two-component systems PhoP-PhoQ and PmrA-PmrB (2, 5–8). Universally, however, these mechanisms involve the reduction of lipopolysaccharide net charges, compromising the binding of cationic polymyxins.

Recently, mobile colistin resistance in the form of the plasmid-mediated *mcr-1* gene was described; this gene encodes a phosphoethanolamine transferase (9). Following its initial discovery, *mcr-1* has been reported worldwide (10). Currently, at least 12 variants of this gene which encode phosphoethanolamine transferase enzymes that differ from *mcr-1* at a single amino acid have been described. Immediately after the discovery of this first gene, other mobile elements significantly different from *mcr-1* have been described: *mcr-2* was found in Belgium, and *mcr-3* was identified in Malaysia, Thailand, and the United States (11, 12). Most recently, *mcr-4* was characterized in an Italian strain of *Salmonella enterica* serovar Typhimurium that was originally isolated in swine in 2013. This gene was also demonstrated to have circulated in the veterinary environment in Belgium and Spain in strains collected in 2015 and 2016 (13). *mcr-5* was instead described in Germany in an isolate of *Salmonella enterica* serovar Paratyphi B, allowing postulation that the transfer of resistance genes from bacterial chromosomes to mobile genetic elements has occurred in multiple independent events (14).

Almost contemporarily with the emergence and dissemination of *mcr-1*, international committees (a EUCAST/CLSI joint working group) sought to address the inconsistencies surrounding the antimicrobial susceptibility testing (AST) of colistin. They concluded that broth microdilution (BMD) should serve as the reference method for testing susceptibility to colistin/polymyxin compounds. Owing to the large size and cationic nature of polymyxins, disk diffusion and gradient diffusion have been demonstrated to be unreliable (15). Additionally, agar dilution is not logistically feasible in clinical settings. Therefore, it follows that the development of a commercial test for colistin AST based on BMD is highly desirable.

Here we present a clinical evaluation of SensiTest Colistin (STC; Liofilchem, Italy), a compact 4-test panel containing the lyophilized antibiotic in 7 2-fold dilutions (0.25 to 16 μg/ml) with one additional well as a growth control. The system is proposed to evaluate colistin susceptibility using a BMD method that complies with the recommendations of international standards (i.e., CLSI, EUCAST) in a simpler and less time-consuming way.

The results of STC were compared with those of the classical BMD technique, performed according to the recommendations of CLSI and EUCAST and used as a "gold standard," and, for some isolates, also with the results of an automated system, the Phoenix 100 system (PHX; Becton Dickinson, USA).

MATERIALS AND METHODS

Strain collections. Three different sets of isolates were analyzed by two different laboratories (the Clinical Microbiology Laboratory, Reggio Emilia, Italy [center A] and the Department of Medical Microbiology and Infectious Diseases, Cardiff, UK [center B]). A total of 353 bacterial strains were evaluated.

Out of the 353 strains, 216 were collected in Italy and analyzed in center A. Of these 216 strains, 159 isolates were prospectively collected from blood culture samples and analyzed as fresh isolates. Fifty-seven of the 216 strains belonged to laboratory collections of peculiar clinical isolates that had been collected in the previous 2 years and stored at −80°C in microbeads (Pro-Lab Diagnostics, USA) and were selected on the basis of the following characteristics: 45 were isolates of the *Enterobacteriaceae* that had reduced susceptibility to colistin (7 of them were *Escherichia coli* isolates carrying the *mcr-1* gene, as demonstrated using an in-house PCR) and 12 were carbapenem-resistant *K. pneumoniae* strains.

Fifty-nine out of the 353 strains were collected over the previous 3 years as part of a clinical study in Tanta Teaching Hospital, Egypt, and were stored in the laboratory in Cardiff at −80°C in microbeads (Pro-Lab Diagnostics). Of these, 20 *Enterobacteriaceae* strains were colistin resistant (according to EUCAST breakpoints) with an undefined mechanism and 39 strains were colistin susceptible. These strains were evaluated in center B.

Seventy-five out of the 353 strains had been collected in Thailand within the last 12 months and were also stored in the laboratory in Cardiff, again, at −80°C using microbeads. All these strains harbored the *mcr-1* gene, which was confirmed by PCR. Also these isolates were analyzed in center B.

Finally, three reference strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. coli* NCTC 13846) were included as controls and tested to validate the different experimental sessions in both laboratories. Details about the isolates analyzed in the present study are shown in Table 1.

STC. (i) Strain analysis. Briefly, a 0.5 McFarland suspension of the microorganism to be tested was prepared in a solution of 0.90% (wt/vol) NaCl (saline) and then diluted 1:20, always in saline, obtaining solution A. Solution A (0.4 ml) was then added to the 3.6-ml tube of Mueller-Hinton broth II provided in the STC kit, obtaining solution B. One hundred microliters of solution B was then dispensed into each well in a row. The STC panels were then incubated at 36 ± 2°C for 16 to 20 h in ambient air. The results were read visually with the naked eye by two different operators, by using bright, indirect lighting against a dark background. The presence of growth in the growth control well was considered first, allowing the test to be considered valid. For MIC determination, bacterial growth was considered the presence of turbidity, a button at the bottom of the well, or pinpoint colonies in the broth. Different lots of the panels were tested during the study period.

(ii) Evaluation of reproducibility and stability. Tests for reproducibility and stability were performed in center A. Six different strains were chosen: the three quality control (QC) reference strains (which included the *E. coli* NCTC 13846 *mcr-1*-positive strain), two *E. coli* strains (one, named CSR-55, with an MIC of 8 μg/ml with an undefined resistance mechanism and the other, named CSR-57, with an MIC of 2 μg/ml), and one carbapenem-resistant *K. pneumoniae* strain with an MIC of ≥16 μg/ml due to mechanisms different from mobile determinants. All the isolates were tested as replicates 10 times.

The stability of the product was assessed in two ways. First, three different boxes containing 32 panels were stored at 4°C (as suggested by the manufacturer), at room temperature, and at 35°C. The six different strains described above were tested on the day after the arrival of the panels (time zero [T0]) and 1 week after (T7), 2 weeks after (T14), 1 month after (T30), and 3 months after (T90) the delivery of the products.

Each single panel allows testing of up to four strains; if less than 4 tests are performed, the manufacturer provides a film with which to seal the inoculated rows (in order to prevent any leakage of contaminated fluids) and to return the panel into its own desiccant envelop, which is placed in a refrigerator. The stability of the panels used at the different times was established by analyzing the six different strains described above with six different panels at day 0 (T0) (inoculum in the first row) and then after 5 (T5), 13 (T13), and 19 (T19) days, in the meantime storing the panels in a refrigerator and then inoculating the second (at T5), third (at T13), and fourth (at T19) rows.

The expected values of the MICs were those established by the EUCAST Routine and Extended Internal Quality Control for MIC Determination and Disk Diffusion (version 7.0, valid from 1 January 2017) for the control strains or a ±1 dilution of the MIC obtained in five previously performed BMD experimental sessions for the other strains.

Broth microdilution. BMD was performed according to the ISO standard method (20776-1), which has been demonstrated to work well for *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* species. Colistin sulfate salt was bought from Sigma-Aldrich (Merck KGaA, Germany) as a lyophilized powder (100 mg, 15,000 U/mg) that was resuspended in distilled water. Vials with a final concentration of 1,024 μg/ml were stored at −80°C until the different test sessions were performed. The cation-adjusted Mueller-Hinton broth (CAMHB) used was ready-for-use Mueller-Hinton II medium (Liofilchem, Italy). The trays bought from Nuova Aptaca, Italy, were made of plain polystyrene and not treated in any way before use. No additives (Tween 80 or other surfactants) were added in any part of the testing process.

For any working session, accounting for 20 isolates, 11 working concentrations of colistin (range, 0.064 μg/ml to 64 μg/ml in 2-fold dilutions) were prepared in separate tubes containing CAMHB, according to the dilution scheme proposed by CLSI (16). Fifty microliters of each intermediate concentration was dispensed into the wells of the microwell plates. For each strain tested, a positive growth control was included in the first well of the plate.

Quality control strains were tested in the first session; each further experimental session was validated by using one of these strains added on rotation.

Isolated bacterial colonies were selected from an 18- to 24-h blood agar culture and transferred to a CAMHB tube. The broth was incubated overnight at 35 to 37°C, the turbidity was adjusted to a 0.5 McFarland standard, and the suspension was then diluted in broth to obtain a final bacterial concentration of 5×10^5 CFU/ml.

Finally, 50 μl of the bacterial suspension was added to each well of the 96-well microplates, which were incubated in an ambient air incubator at 35°C ± 2°C for 16 to 20 h. The MICs were determined as the lowest concentration that completely inhibited the bacterial growth in the wells.

Phoenix 100 system. The tests with the Phoenix 100 system were performed in center A, according to the manufacturer's recommendations as a part of the standard of care for the patients from whom the 216 clinical isolates were obtained. PHX was also performed on the 3 QC strains, for a total of 219 isolates tested. The NMIC-417 card was used for the present study.

Discrepant results. Samples with discrepant results that showed a disagreement between BMD and STC with more than a 2-fold dilution were retested, with both methods being performed in at least two other different experimental sessions. The results of each test that were confirmed in more than two experimental sessions were considered confirmed, and the MIC value was used for the final analysis.

Agreement between methods. Essential agreement (EA) was defined as the agreement within plus or minus 1 2-fold dilution of the MIC determined by STC with the MIC determined by the reference method (BMD). EA determination was evaluated exactly for all the values below 16 μg/ml (values of ≥16 μg/ml were considered in agreement). EA between PHX and BMD was not analyzed due to the narrow MIC range tested by the PHX card (only 3 dilutions from 1 to 4 mg/liter).

TABLE 1 Study results[a]

Strain	Total no. of isolates (no. colistin resistant[b])	No. of isolates for which MIC was:		% EA	No. (%) of strains for which the following were obtained:		
		Same	±1 dilution		CA	ME	VME
Acinetobacter baumannii	6 (0)	1	5	100.0	6 (100)	0	0
Acinetobacter species[c]	4 (1)	3	1	100.0	4 (100)	0	0
Citrobacter koseri	1 (0)	0	1	100.0	1 (100)	0	0
Enterobacter aerogenes	4 (0)	1	1	50.0	3 (75.0)	1	0
Enterobacter cloacae complex	4 (2)	1	3	100.0	4 (100)	0	0
Escherichia coli	205 (89)	80	119	97.1	204 (99.5)	0	1
Hafnia alvei	6 (6)	3	2	83.3	6 (100)	0	0
Klebsiella oxytoca	6 (0)	2	4	100.0	6 (100)	0	0
Klebsiella pneumoniae	76 (23)	34	38	94.7	75 (98.7)	1	0
Leclercia adecarboxylata	1 (0)	0	1	100.0	1 (100)	0	0
Morganella morganii	2 (2)	2	0	100.0	2 (100)	0	0
Proteus mirabilis	5 (5)	5	0	100.0	5 (100)	0	0
Providencia species	2 (2)	2	0	100.0	2 (100)	0	0
Pseudomonas aeruginosa	19 (0)	9	10	100.0	19 (100)	0	0
Salmonella species	7 (2)	3	3	85.7	6 (85.7)	0	1
Serratia marcescens	4 (4)	4	0	100.0	4 (100)	0	0
Shigella species	1 (1)	1	0	100.0	1 (100	0	0
Total	353	151	188	96.0	349 (98.9)	2	2
Colistin susceptible	216	81	124	94.9	214 (99.1)	2	NA
Colistin resistant (not due to *mcr-1*)	54	43	9	96.3	53 (98.1)	NA	1
Colistin resistant (due to *mcr-1*)	83	27	55	98.8	82 (98.8)	NA	1
Total	353	151	188	96	349 (98.9)	2	2

[a]EA, essential agreement; CA, categorical agreement; ME, major errors; VME, very major errors; NA, not applicable.
[b]Colistin resistance was defined according to EUCAST breakpoints.
[c]These included one strain each of *Acinetobacter ursingii*, *A. lwoffii*, *A. junii*, and *A. nosocomialis*.

For discrepant results, we evaluated the categorical agreement (CA), i.e., when the results did not change the categorization of the isolates (considered susceptible or resistant) or the occurrence of major errors (ME; the BMD result was susceptible and the STC or the PHX result was resistant) or very major errors (VME; the BMD result was resistant and the STC or the PHX result was susceptible) (17).

RESULTS

The study results are summarized in Table 1. All the tests performed were considered valid (that is, growth was present in the growth control well). Only in a few cases was the evaluation of growth challenging, due to the presence of pinpoint colonies scattered into the broth. That occurred mainly for *Hafnia alvei* strains. Examples of the STC panels are shown in Fig. 1.

Agreement between STC and BMD. Essential agreement (EA) was obtained for 339 out of the 353 strains tested (96.0%). In particular, for 151 strains the same MIC value was obtained by the two methods, whereas a difference of ±1 dilution was documented for 188 isolates.

Discrepancies of 2 or more dilutions were documented for 14 strains: 6 strains from the Italian clinical collection, 7 strains from those collected in Egypt, and 1 *mcr-1* isolate collected in Thailand. These included 6 *Escherichia coli* isolates, 4 *K. pneumoniae* isolates, 2 *Enterobacter aerogenes* isolates, 1 strain of *H. alvei*, and 1 isolate of *Salmonella* species. The discrepancy did not change the strains' clinical categorization in 12 of these cases; the other 2 cases were 1 isolate of *Enterobacter aerogenes* (MICs, 0.25 μg/ml by BMD and 4 μg/ml by STC) and one *Salmonella* species (MICs, 4 μg/ml by BMD and 1 μg/ml by STC). Overall categorical agreement (CA) was obtained for 349 out of the 353 strains analyzed (98.9%). Two MEs were recognized for one strain of *E. aerogenes* (as described above) and one strain of *K. pneumoniae* (MICs of 2 μg/ml by BMD and 4 μg/ml by STC), whereas two VMEs were documented for one strain each of *Salmonella* species (as described above) and *E. coli* (MICs, 4 μg/ml by BMD and 2 μg/ml by STC). The rate of MEs for STC was 0.92% (2 isolates out of the 216 susceptible strains), whereas the rate of VMEs was 1.46% (2 out of 137 isolates).

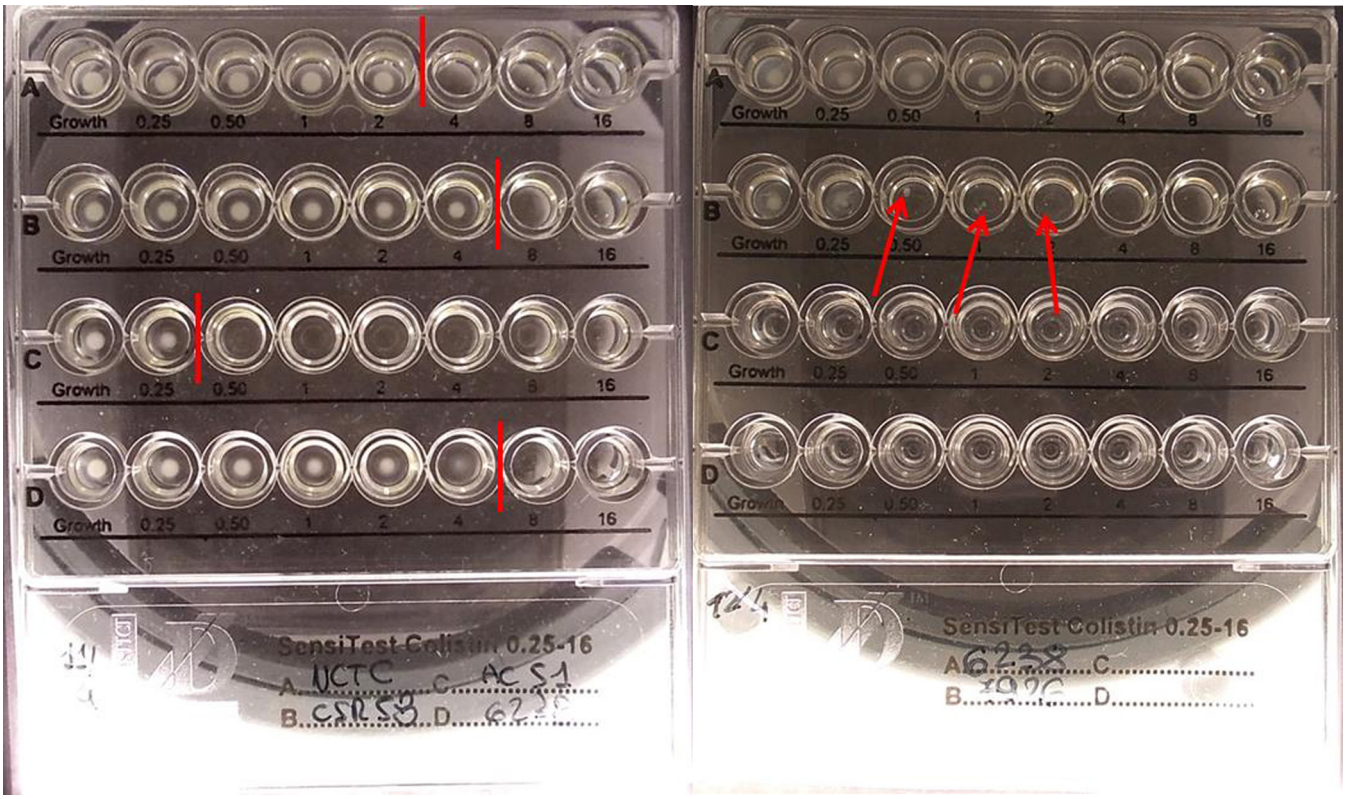

FIG 1 (Left) Results of an STC test. The well on the right of the red line indicates the value of the MIC for the isolate. (Right) Results of another STC test. Arrows, pinpoint colonies of *H. alvei*.

Reproducibility of STC. The six strains gave a total of 60 replicates. Total agreement among the replicates (i.e., the same MIC) was documented in 50 out of the 60 tests performed (83.3%), whereas the EA was 100%.

Stability of STC. Stability tests on the STC panels were performed across different time periods with the STC panels stored at different temperatures, as shown in Table 2. All the tests fell into agreement within ±1 2-fold dilution. Only one was out of range: the MIC at T7 of the reference strain NCTC 13846 (*E. coli* harboring the *mcr-1* gene) for the panels stored at 4°C, which was 2 dilutions higher than expected.

The STC also appeared to be stable if MICs were evaluated at different times for the different rows of the same panel (Table 3).

Agreement between BMD and PHX. PHX showed an overall agreement with BMD for 212 out of the 219 isolates analyzed (96.8%). The seven strains with discordant results resulted in one ME for a *P. aeruginosa* strain and six VMEs (two for *E. coli* [one strain harboring the *mcr-1* gene], two for *Salmonella* species, one for an *H. alvei* strain, and one for a *K. pneumoniae* strain), accounting for 10% of the 60 colistin-resistant isolates tested.

DISCUSSION

Colistin is often considered the last resource for the treatment of severe Gram-negative bacterial infections, in particular, those caused by *P. aeruginosa*, *A. baumannii*, and CRE (3). This compound has no activity against Gram-positive bacterial strains or against some Gram-negative bacteria, such as *Burkholderia cepacia*, *Elizabethkingia meningoseptica*, *H. alvei*, *Morganella morganii*, *Proteus* species, *Providencia* species, *Serratia marcescens*, and *Yersinia pseudotuberculosis* (2, 18, 19).

Immediately after the recent discovery of a plasmid-mediated mechanism of resistance that more often conveys low-level resistance (MICs, 4 to 8 μg/ml) (9), strains (mostly *E. coli*) harboring the gene have been described worldwide (10, 20). More recently, other mobile genetic resistance traits have been described (11–14). Thus, the

TABLE 2 Stability tests using panels stored at different temperatures for 3 months

Panel storage temp and strain	MIC (μg/ml)					
	Expected range	T0	T7	T14	T30	T90
4°C						
ATCC 25922	0.25–2[a]	1	0.5	0.25	0.5	0.5
ATCC 27853	0.5–4[a]	1	1	1	1	1
CSR-55	4–16	16	8	8	8	8
CSR-57	1–4	4	4	2	4	4
CSR-68	8–32	≥16	≥16	≥16	≥16	≥16
NCTC 13846	2–8[a]	4	16	4	4	8
Negative control		NG[b]	NG	NG	NG	NG
Room temp						
ATCC 25922	0.25–2[a]	0.5	0.5	0.5	1	1
ATCC 27853	0.5–4[a]	1	1	1	1	1
CSR-55	4–16	8	16	8	16	8
CSR-57	1–4	4	4	2	4	4
CSR-68	8–32	≥16	≥16	≥16	≥16	≥16
NCTC 13846	2–8[a]	4	4	4	4	4
Negative control		NG	NG	NG	NG	NG
35°C						
ATCC 25922	0.25–2[a]	0.5	0.5	0.5	1	1
ATCC 27853	0.5–4[a]	1	1	1	1	2
CSR-55	4–16	8	8	8	8	≥16
CSR-57	1–4	4	4	4	4	4
CSR-68	8–32	≥16	≥16	≥16	≥16	≥16
NCTC 13846	2–8[a]	4	4	4	4	8
Negative control		NG	NG	NG	NG	NG

[a]According to EUCAST Routine and Extended Internal Quality Control for MIC Determination and Disk Diffusion (version 7.0, valid from 1 January 2017).
[b]NG, no growth.

spread of colistin-resistant microorganisms has become a matter of concern worldwide, and the ECDC published in June 2016 a document calling for a rapid risk assessment in order to control the spread of plasmid-mediated colistin resistance in *Enterobacteriaceae* (https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/enterobacteriaceae-risk-assessment-diseases-caused-by-antimicrobial-resistant-microorganisms-europe-june-2016.pdf). This document highlights different actions for the implementation of surveillance strategies and antimicrobial stewardship about this topic. Moreover, the development of improved laboratory methods for the determination of the correct colistin MIC and molecular *mcr-1* detection is considered beneficial and necessary (21).

For colistin, a reliable evaluation of MICs, together with the possibility of therapeutic drug monitoring (TDM), is also necessary for the correct management of patients, given colistin's potential significant adverse effects, in order to avoid toxic effects while maintaining sufficient antibacterial activity (21).

TABLE 3 Stability tests using the same panel inoculated on different days

Strain	MIC (μg/ml)				
		Single-panel replicates			
	Expected range	T0	T5	T13	T19
ATCC 25922	0.25–2[a]	0.5	0.5	1	1
ATCC 27853	0.5–4[a]	1	1	2	2
CSR-55	4–16	16	8	8	8
CSR-57	1–4	4	4	4	4
CSR-68	8–32	≥16	≥16	≥16	≥16
NCTC 13846	2–8[a]	4	4	4	4

[a]According to the EUCAST Routine and Extended Internal Quality Control for MIC Determination and Disk Diffusion (version 7.0, valid from 1 January 2017).

A joint EUCAST and CLSI subcommittee issued recommendations in July 2016 (subsequently confirmed in updates of August and November 2016 and June 2017) confirming that broth microdilution, using untreated polystyrene, is so far the only valid method and that disk diffusion does not work because of the poor diffusion of the large colistin molecule (15, 22). In this document, the adhesive properties related to the cationic nature of colistin were emphasized, suggesting that the BMD test should be performed by using cation-adjusted Mueller-Hinton broth without additives (in particular, no polysorbate 80 or other surfactants) with trays of untreated polystyrene. Colistin may adhere to BMD plates; this issue should be taken into account when developing new devices. In cases of major discrepancies with the result expected, verification of the free concentrations of colistin in the test panels may be warranted. The trays used to produce the STC panels are made of plain, untreated polystyrene, compliant with EUCAST-CLSI guidelines. The results obtained in our various STC experiments were congruent with those of BMD, and so it was decided that evaluation of the free concentrations of colistin in these cases was unnecessary.

Moreover, issues about the correct MIC estimate with some automated instruments have been recently highlighted. EUCAST suggests to the users of semiautomated devices to apply rigorous QC, checking with the manufacturer whether or not they are confident that their method to evaluate colistin gives correct results (22). Regarding automated systems, the performances of the Vitek-2 (bioMérieux, Marcy l'Etoile, France) and MicroScan (Beckman Coulter, CA, USA) systems against CRE and *mcr-1*-positive isolates have recently been evaluated, resulting in a high rate of VMEs for Vitek-2 (36% versus 4% for MicroScan), whereas no MEs were documented for Vitek-2 and 15.8% MEs were demonstrated for MicroScan (23). The high rate of VMEs with the Vitek-2 AST-GN colistin (cs01n) system compared to the results of agar dilution (the reference method used for cs01n development) and compared to those of BMD was also demonstrated by an internal investigation performed by bioMérieux (bioMérieux, communication to customers).

These data express the pertinence of the development of a new diagnostic tool for clinical microbiology laboratories. Nevertheless, standard BMD methods are labor-intensive and do not fall in the routine practice of the majority of the clinical microbiology laboratories.

SensiTest Colistin appears to be a simple but highly reliable test to assess colistin susceptibility. The preparation procedure is easy and fast, and evaluation of the results is simple, reflecting that of the BMD methodology. With this device, it is possible to test a single drug, and therefore, it may be used as a second-line assay for laboratories which use automated instruments in their daily practice without redundancies for other antibiotics. Even if the panels are customized for testing four separate isolates at distinct time points, the MIC given by STC appeared to be stable.

In our assessment, the device appeared to be highly reproducible. We tested the reproducibility of the panels stored at different temperatures, reflecting conditions that the STC panels may experience in real-life use under particularly challenging conditions, such as those that may occur in developing countries, with possible troubleshooting due to transport and storage.

As an ancillary result of our study, we demonstrated an overall good agreement between BMD and the Phoenix 100 system, even with the limitation that PHX was performed as the standard of care a few days before the execution of the BMD. Among the 219 isolates also tested with PHX, the VMEs involved 2 strains each of *E. coli* (1 *mcr-1*-positive isolate) and *Salmonella* species, 1 strain of *H. alvei*, and 1 strain of *K. pneumoniae*. The rate of VMEs for PHX was 10% in our study (6 isolates out of the 60 colistin-resistant strains analyzed with the automated instrument).

STC was recently evaluated by the EUCAST committee in June 2017, together with other commercially available BMD techniques. In that study, STC was tested on 75 strains: the EA appeared to be 88%, with 7 MEs and 1 VME (22). In our study, the test performance was better, with EA of 96.0% (2 MEs and 2 VMEs). A direct comparison between these data and those from our study appears to be difficult because of a likely

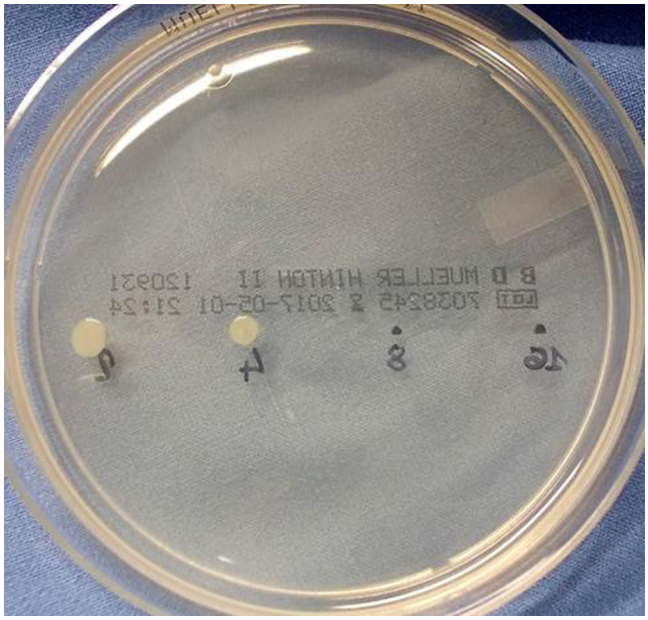

FIG 2 Evaluation of the bactericidal effect by spotting 1 μl of the resuspended wells of a strain having an MIC of 8 mg/ml (the MIC is equal to the MBC).

disparity between the EUCAST strains tested and our clinically representative, geographically varied test panel.

In our experience, STC showed a very good performance with strains carrying the *mcr-1* gene, with 98.8% EA (Table 1). For some strains, such as *H. alvei*, it is important to carefully evaluate any growth in the wells, as it may present as pinpoint microcolonies, as was the case for this species (Fig. 1). However, it is likely that the recent caveat that this microorganism be reclassified as constitutively resistant to colistin (19) may render in future the evaluation of colistin susceptibility for these strains redundant.

Another possible advantage of using STC is that, being an open system, it is possible to evaluate also the minimal bactericidal concentration (MBC) after the reading is obtained by spotting 1 to 10 μl onto a Mueller-Hinton agar plate (Fig. 2) and considering the growth after 24 h.

In the present study, a limited number of *A. baumannii* and *P. aeruginosa* strains were tested, and further evaluation with these isolates may be of value. However, even if further assessments, perhaps through in-field/in-clinic studies, may be warranted, our preliminary findings suggest that STC could be proposed both as a first-line test for selected specimens and as a confirmatory test adjacent to automated screening.

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We have no conflicts of interest to declare.

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