Abstract: A homemade and culture-based test, relying on the visual detection of the reduction of the resazurin reagent (a cell viability indicator), has been developed for the rapid detection of polymyxin resistance in *Acinetobacter baumannii*. Here, we evaluated the industrial version of this test, the RapidResa Polymyxin *Acinetobacter NP®* test (Liofilchem, Italy). A well-characterized panel of 68 clinical *A. baumannii* strains (36 polymyxin-susceptible, 26 polymyxin-resistant *A. baumannii*, and 6 colistin-heteroresistant isolates) of worldwide origin was tested. All the colistin-susceptible *A. baumannii* isolates gave positive results according to the RapidResa Polymyxin *Acinetobacter NP®* test, except for a single isolate that gave a false-positive result. Out of the 26 colistin-resistant *A. baumannii* strains, 25 were correctly identified as colistin resistant using the RapidResa Polymyxin *Acinetobacter NP®* test. Only a single colistin-resistant *A. baumannii* strain gave a false-negative result. Additionally, the six colistin-heteroresistant isolates tested gave positive results. Altogether, the sensitivity and the specificity of the test were found to be 96% and 97%, respectively. The turn-around-time of this easy-to-perform test was 3–4h, which showed excellent reliability for identification of polymyxin resistance in *A. baumannii*. The RapidResa Polymyxin *Acinetobacter NP®* test allows a rapid differentiation between polymyxin-susceptible and -resistant *A. baumannii* isolates, which may contribute to optimization of the use of polymyxins for treating infections due to multidrug-resistant *A. baumannii*. 

Keywords: colistin; *Acinetobacter*; susceptibility testing; diagnostic test

1. Introduction

Multidrug resistance in *Acinetobacter baumannii* is becoming a source of major concern worldwide [1]. This opportunistic pathogen is intrinsically resistant to many antibiotic agents and can also acquire resistance to all antibiotics, virtually. Life-threatening nosocomial infections associated with these multidrug-resistant (MDR) species are increasingly reported, particularly in patients admitted to intensive care units, and may lead to increased mortality rates due to limited therapeutic options [2–4]. Very high percentages of carbapenem-resistant *A. baumannii* have been observed worldwide during the past decade [1,5]. Therefore, polymyxins, such as colistin, are now considered as last resort antibiotics for the treatment of infections with carbapenem-resistant *A. baumannii* (CRAB) [6–8]. Currently, colistin resistance is a serious global concern due to limited alternative antibiotics [9,10]. Owing to the global increasing occurrence of colistin resistance
and heteroresistance in this species, the rapid detection of colistin susceptibility is critical to optimally adapt the empirical treatment [11]. Choice of a polymyxin-based treatment is mainly based on the result of susceptibility testing performed through different techniques. The antimicrobial susceptibility testing for colistin is technically challenging [12,13]. The current standard method of detection for polymyxin susceptibility in Gram negatives is the determination of minimum inhibitory concentration (MIC) by the broth dilution (BMD) method (https://eucast.org/clinical_breakpoints/ accessed on 6 January 2021). However, this procedure is time consuming (24 h), and not easy to implement for most clinical laboratories [14].

Recently, the Rapid ResaPolymyxin Acinetobacter NP test has been developed [15]. This culture-based technique allows categorization between colistin-susceptible/-resistant A. baumannii isolates in 4 h. It is based on the utilization of resazurin (7-hydroxy-3H-phenoxazin-3 one 10-oxide), also referred to as the alamarBlue® and the prestoBlue®, by growing bacteria. Its principle is based on the fact that metabolically active cells reduce the blue resazurin to the pink product resorufin. This reduction is proportional to the number of metabolically active cells. Detection of the blue/pink color of the wells is made visually and does not require specialized equipment. Bacterial viability of A. baumannii isolates, after growth in medium with or without a defined concentration of colistin, is therefore evidenced [15]. The main value of this test is rapid categorization of susceptibility and resistance, which is important to optimally adapt the empirical treatment [16,17].

A commercial version of this test, the RapidResa Polymyxin Acinetobacter NP® test (Liofilchem, Roseto degli Abruzzi, Italy), is now available, based on the same protocol as its homemade version. This test complements the Rapid Polymyxin NP® test, which works with Enterobacterales but is not appropriate for non-fermenters, such as A. baumannii [18]. The aim of our present study was to evaluate this novel commercial test using a collection of polymyxin-susceptible and polymyxin-resistant A. baumannii isolates by comparison with the gold standard method for colistin susceptibility testing and to determine the sensitivity and the specificity of this commercial test.

2. Materials and Methods

A well-characterized panel of 68 clinical A. baumannii strains (36 polymyxin-susceptible, 26 polymyxin-resistant A. baumannii, and 6 colistin-heteroresistant isolates) of worldwide origin (either from colonization or true infections) was used in this study. The colistin-resistant A. baumannii R3402 strain (positive control) and the colistin-susceptible A. baumannii R4 strain (negative control) were used. The polymyxin susceptibility or resistance of the strains was determined by the precise determination of MIC values using the broth dilution (BMD) method. Isolates were considered as susceptible when MICs of colistin were ≤2 mg/L and resistant when MICs were >2 mg/L (http://www.eucast.org/clinical_breakpoints/, accessed on 25 December 2020).

The RapidResa Polymyxin Acinetobacter NP® assay was performed according to the manufacturer’s recommendations from fresh overnight bacterial colonies grown at 37 ºC on non-selective agar plates. UriSelect 4 agar plates (Bio-Rad, Marnes-la-Coquette, France) were used in this study for overnight cultures of A. baumannii isolates. Briefly, isolated colonies were suspended from an overnight agar plate into a vial of Mueller-Hinton II broth provided in the kit. The suspension was adjusted to obtain a turbidity equivalent to 0.5 McFarland standard. Therefore, the suspension contained approximatively 1.2 × 10^8 CFU/mL that was used within 15 min. Then, 200 µL of Mueller-Hinton II broth were added to well a (as sterility control). Then, 200 µL of isolate suspension (tested isolate) were added in wells b, c (Figure 1). Then, 20 µL of the resazurin solution (provided in the kit) were added to each well in a single column. The reagent was mixed with the medium by pipetting up and down. The panels were then covered with the lid provided and incubated for up to 3–4 h in ambient air at 36 ± 1 ºC and the color of the well inspected every 1 h (results are mostly obtained after 3 h of incubation). Colistin-resistant isolates grew in the absence and the presence of colistin (wells b and c) (i.e., purple or pink, indicating
polymyxin resistance) while colistin-susceptible isolates grew only in wells b that did not contain any colistin (i.e., purple or pink) but not in wells c (i.e., blue, indicating polymyxin susceptibility) (Figure 1). Such a panel allows testing of eight different isolates.

3. Results and Discussion

Among the 62 A. baumannii isolates tested to evaluate the performance of the RapidResa Polymyxin Acinetobacter NP® test, 36 were colistin susceptible (MICs of colistin ranging from <0.125 to 1 mg/L) and 26 isolates were resistant (MICs of colistin ranging from 4 to ≥128 mg/L), according to the results of the BMD method (Table 1). The 36 colistin-susceptible A. baumannii isolates gave negative results according to the RapidResa Polymyxin Acinetobacter NP® test, except for a single isolate (MICs of 0.25 mg/L), which gave false-positive results (Table 1). Out of the 26 colistin-resistant A. baumannii strains, 25 strains were correctly identified as colistin resistant using the RapidResa Polymyxin Acinetobacter NP® test. Only one colistin-resistant A. baumannii strain gave a false negative result. Additionally, six colistin heteroresistant isolates were also tested, which gave positive results (indicating colistin resistance). The specificity (which measures the proportion of negatives that are correctly identified) was calculated at 96%, and the sensitivity (which measures the proportion of positives that are correctly identified) was calculated at 97%.
Table 1. RapidResa Polymyxin Acinetobacter NP® test for rapid detection of polymyxin resistance in *A. baumannii* isolates.

| Strain | Origin          | Phenotype | BMD MIC Colistin (mg/L) | RapidResa Polymyxin Acinetobacter NP® Test Result | Discrepancy with BMD MIC Colistin Result |
|--------|-----------------|-----------|-------------------------|-----------------------------------------------|----------------------------------------|
| R3     | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R4     | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R5     | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R6     | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R8     | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R11    | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R14    | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R16    | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R21    | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R27    | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R32    | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R34    | France Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R35    | France Environmental | S     | <0.125                  | Negative                                      | -                                      |
| R2536  | Portugal Clinical | S     | 0.5                     | Negative                                      | -                                      |
| R3381  | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| R3382  | Turkey Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R3383  | Turkey Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R3384  | Turkey Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R3385  | Turkey Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R3386  | Turkey Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R3387  | Turkey Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R3389  | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| R3391  | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| R4319  | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| R4321  | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| R4323  | Switzerland Clinical | S    | 1                       | Negative                                      | -                                      |
| R4685  | Lybia Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R4687  | Lybia Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R4692  | Lybia Clinical | S         | <0.125                  | Negative                                      | -                                      |
| R1401  | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| R854   | Greece Clinical | S         | <0.125                  | Negative                                      | -                                      |
| N581   | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| N5891  | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| N211   | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| N223   | Switzerland Clinical | S    | <0.125                  | Negative                                      | -                                      |
| N3390  | Switzerland Clinical | S    | 0.25                    | Positive                                      | Yes                                    |
| R3393  | Italy Clinical | R         | 8                       | Positive                                      | -                                      |
| R3395  | Italy Clinical | R         | 64                      | Positive                                      | -                                      |
| R3396  | Spain Clinical | R         | 4                       | Positive                                      | -                                      |
| R3397  | Spain Clinical | R         | 16                      | Positive                                      | -                                      |
| R3398  | Switzerland Clinical | R    | 128                     | Positive                                      | -                                      |
| R3399  | Turkey Clinical | R         | 16                      | Negative                                      | Yes                                    |
| R3400  | Turkey Clinical | R         | 8                       | Positive                                      | -                                      |
| R3401  | Turkey Clinical | R         | 32                      | Positive                                      | -                                      |
| R3402  | Turkey Clinical | R         | 32                      | Positive                                      | -                                      |
| R3403  | Turkey Clinical | R         | >128                    | Positive                                      | -                                      |
| R3404  | Turkey Clinical | R         | 4                       | Positive                                      | -                                      |
| R3405  | Turkey Clinical | R         | >128                    | Positive                                      | -                                      |
| R3557  | Switzerland Clinical | R    | 32                      | Positive                                      | -                                      |
| R3804  | Czech Republic Veterinary | R    | 16                      | Positive                                      | -                                      |
| R3811  | Switzerland Clinical | R    | 16                      | Positive                                      | -                                      |
| R4322  | Switzerland Clinical | R    | >128                    | Positive                                      | -                                      |
| R4326  | Switzerland Clinical | R    | 16                      | Positive                                      | -                                      |
| R4327  | Switzerland Clinical | R    | 128                     | Positive                                      | -                                      |
| R4330  | Switzerland Clinical | R    | >128                    | Positive                                      | -                                      |
In another study, Kon et al. evaluated the performance of another commercial kit (Rapid Polymyxin™ Acinetobacter (RP-AB) (ELITechGroup, Puteaux, France) for colistin susceptibility testing [19]. The Rapid Polymyxin™ Acinetobacter (ELITechGroup) is based on the colorimetric detection of glucose metabolism associated with bacterial growth resulting in a color change (red to yellow) of the pH indicator (phenol red) while the present test, the RapidResa Polymyxin Acinetobacter NP® test (Liofilchem), is based on the reduction of the blue resazurin to the pink product resorufin by metabolically active bacteria cells. Unlike the RapidResa Polymyxin Acinetobacter NP® test (Liofilchem), the results of RP-AB (ELITechGroup) were difficult to interpret and poorly correlated with the results of the BMD method, resulting in an unacceptable rate of very major errors. It showed a sensitivity and specificity of 41.2% and 86.1%, respectively [19]. Previous studies have evaluated the performance of the homemade test, the RapidResaPolymyxin NP test, using a collection of A. baumannii clinical isolates [16,17]. The sensitivity and specificity of the Rapid ResaPolymyxin NP test compared to the broth microdilution method was 100 and 96%, respectively, suggesting that this test is highly sensitive and specific for the detection of colistin resistance in A. baumannii [16]. In another study, the Rapid ResaPolymyxin NP test showed a 95.1% categorical agreement with results of the standard broth microdilution method [17].

Compared to Rapid Polymyxin™ Acinetobacter (ELITechGroup), the RapidResa Polymyxin Acinetobacter NP® test (Liofilchem) showed an excellent concordance with the results of the susceptibility test (BMD), for susceptible and resistant isolates.

4. Conclusions and Future Perspective

This study showed that the RapidResa Polymyxin Acinetobacter NP® test (Liofilchem) is a reliable technique for detecting polymyxin resistance in A. baumannii. Further studies may further analyze the efficacy of this test to rapidly detect colistin resistance among additional clinically significant Acinetobacter spp. This test is rapid and easy to implement in any laboratory. It offers the possibility of detecting polymyxin resistance from bacterial cultures in 3–4 h. Although the BMD method requires an additional 18 h for obtaining the results, results of the RapidResa Polymyxin Acinetobacter NP® can be obtained on the same day. Therefore, the use of this test may contribute to optimization of the antibiotic stewardship of infections due to multidrug- or pandrug-resistant A. baumannii strains that are of increasing frequency worldwide.

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