UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

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POLYMYXIN NP ELUTION: A RAPID AND ACCURATE METHODOLOGY TO DETERMINE SUSCEPTIBILITY TO POLYMYXINS AMONG *ENTEROBACTERALES*

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Trabalho de Conclusão de Curso apresentado como requisito parcial para obtenção do grau de farmacêutico pelo curso de Farmácia da Universidade Federal do Rio Grande do Sul.

Orientadora Profª. Dra. Juliana Caierão

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Este trabalho foi elaborado em formato de artigo científico, de acordo com as normas da Revista *Microbial Drug Resistance*. Tais normas foram anexadas ao final do documento, para uma melhor leitura e compreensão da banca avaliadora.

Polymyxin NP elution: a rapid and accurate methodology to determine susceptibility to polymyxins among *Enterobacterales*

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ABSTRACT

Fast and accurate detection of polymyxins resistance has become necessary as they remain one of the few last resources to treat infections caused by Carbapenem-resistant *Enterobacterales* in many regions. We described NP macroelution and established the miniaturized version, NP microelution, aiming to detect polymyxins resistance quickly, accurately and at low cost among *Enterobacterales*. The methodologies consist of exposing bacterial populations of 10^8 CFU/mL in NP solution where polymyxin B disks were previously eluted, reaching concentrations of 2µg/mL for macro and 3µg/mL for NP microelution. Eighty-four *Enterobacterales* isolates were evaluated, 39 (46.4%) resistant to polymyxin B. When compared to broth microdilution (BMD), the NP macroelution obtained 2.4% major error (ME), with specificity of 95.6%, while its miniaturized version presented a slightly lower a ME (1.2%) and higher specificity (97.8%). Both methodologies presented sensitivity of 100.0%, and needed 3 hours incubation to identify over 90% of truly resistant isolates. NP macro and microelution proved to be excellent alternatives to determine polymyxin B susceptibility in routine of microbiology laboratories, presenting low cost, being easy to perform, and demanding short incubation time.

INTRODUCTION

Antimicrobial resistance is currently one of the greatest threats to public health worldwide. Carbapenem-resistant *Enterobacterales* (CRE) are categorized as "priority 1: critical" in the list of most threatening pathogens to human health, established by the World Health Organization (WHO).[1] Infections caused by CRE present, in general, limited therapeutic options and are associated with prolonged length of hospital stays, and high mortality rates .[2–4]

Drugs such as polymyxins and the new combinations of β-lactams and β-lactamase inhibitors, such as ceftazidime-avibactam, are part of the limited therapeutic arsenal available

to treat infections caused by CRE.[3] However, due to the high cost of these combinations, their unavailability in some countries and the inefficiency of avibactam against metallo-β-lactamases, polymyxins-centered therapeutic regimens remain the last resort in many regions.[5,6]

Historically, the clinical use of polymyxins (colistin and polymyxin B) had been abandoned in the 1970s due to their toxic potential. However, about 20 years later, with the emergence of multidrug-resistant Gram-negative bacilli (MDR), their use were re-evaluated, placing them back in the therapeutic pipeline.[7,8]

With this widespread use of polymyxins in human medicine in recent years, associated with the practically uninterrupted use in animal production, the emergence and dissemination of resistance to these drugs have been observed in several countries around the world. Although resistance rates remain low in some countries, there is a steady increase in others, coinciding with the increase in frequency of isolation of CRE.[9,11,13,15] In Brazil, Sampaio & Gales (2016) demonstrated an increase in polymyxin B resistance among KPC-producing *K. pneumoniae* of 27.1% over a period of 4 years.[17]

In this context, rapid and accurate detection of polymyxins resistance is essential for both epidemiological monitoring and therapeutic management.[10] However, because of physicochemical characteristics of polymyxins molecules, such as their size and cationic nature, determining susceptibility “in vitro” is challenging. Conventional techniques routinely used in microbiology laboratories, such as disk-diffusion and concentration gradient strips, are not recommended for this purpose due to high rates of false susceptibility.[7,12,14,16] Indeed, the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) established broth microdilution (BMD) as the reference methodology to determine polymyxins resistance.[18,19] Despite the reliability of results generated by BMD, there are important disadvantages, such as high cost (because of the need

for powdered antibiotics), a relative complexity of execution and, mainly, the requirement for a prolonged incubation (16-20h).[16]

Thus, new methodologies that can be better adapted to clinical microbiology laboratories have been developed. In 2016, Nordmann, Jayol & Poirel[20] described a rapid phenotypic test (Rapid Polymyxin NP, RPNP), able to detect polymyxins resistance in up to 4 hours, based on glucose metabolism by microorganisms in the presence of a defined concentration of antibiotic. The test was broadly and globally evaluated, with sensitivity and specificity ranging from 90 to 100% and 70 to 100%, respectively.[21–30] However, despite its advantages, RPNP is not endorsed by CLSI or EUCAST, and also requires the use of powder antibiotic, increasing costs.

In 2019, Simner and colleagues[31] developed the Colistin Broth Disk Elution (CBDE), which became recommended by CLSI[19] for the detection of colistin resistance, based on data generated by a multicenter study performed by Humphries *et al.* (2019).[32] The methodology is based on the elution of the antibiotic disk content into a broth culture medium, where a standardized bacterial inoculum is added. Growth is indicated by the turbidity of the medium after 16 to 20h of incubation. So far, few publications evaluated this technique, but studies demonstrate good results.[31–33] It should be noted that only our research group (Cielo *et al*., 2020)[33] have tested this methodology using polymyxin B instead of colistin so far.

The main advantage of CBDE is the use of antibiotic disk instead of powder, which considerably reduces costs. On the other hand, the required incubation is as long as BMD, which is a notable disadvantage.

In 2021, Ngudsuntia *et al.*[34] proposed a modification in RPNP, presenting a methodology that determines colistin resistance after elution of a colistin disk in 2.7 mL of NP solution, the rapid colistin disk elution (RCDE), providing results in 4h. RCDE had satisfactory results, encouraging other studies.

Indeed, an alternative methodology that could combine the advantages of RPNP and CBDE would be valuable for clinical microbiology laboratories. Here, we describe the NP macroelution, which is based on RCDE with modifications aiming to improve this methodology. Also, we established the miniaturized version, NP microelution, to detect resistance to polymyxins quickly, accurately and in a low-cost manner.

MATERIALS AND METHODS

Bacterial strains

Eighty-four carbapenem-resistant *Enterobacterales* (74 *Klebsiella pneumoniae*, 3 *Escherichia coli*, 3 *Enterobacter cloacae*, 3 *Klebsiella oxytoca* and 1 *Serratia marcescens*) recovered from clinical specimens of patients attended in three hospitals of Porto Alegre city, Southern Brazil, were used. The study was approved by the local Research Ethics Committee. *E. coli* ATCC 25922 and *Morganella morganii* (intrinsically resistant to polymyxins, MIC >64 μg/mL) were used as negative (sensitive) and positive (resistant) controls, respectively, for all tests that were performed.

Determination of Minimum Inhibitory Concentration (MIC)

To determine polymyxin B MIC, all isolates were submitted to BMD, and results were interpreted according to CLSI[19] guidelines: MIC ≥4 μg/mL indicated resistance.

Polymyxin B Disk Elution (PBDE) and Rapid Polymyxin NP Test (RPNP)

All isolates were submitted to PBDE and RPNP. PBDE was performed as described by Simner *et al*. (2019)[31], with only one modification: the use of polymyxin B instead of colistin. Briefly, each isolate was evaluated in two tubes (the growth control and the test tube) containing 15 mL of cation-adjusted Mueller-Hinton broth (Sigma-Aldrich, USA). In the test tube, it was added a 300 IU polymyxin B (Oxoid, United Kingdom) disk, reaching an antibiotic concentration of 2 μg/mL after elution. This tube was kept at room temperature for 30 minutes to allow elution of polymyxin B from the disk into the broth, before inoculation of a

standardized bacterial suspension (0.5 McFarland). After incubation at 35°C for 16-20h, the isolate was considered positive if turbidity was observed in both tubes.

RPNP was performed according to Nordmann, Jayol & Poirel (2016)[20], in 96-well plates, in which 50 µL of standardized inoculum (3.0 - 3.5 McFarland) were added to 150 µL of NP solution containing 3.75 µg/ml of polymyxin B. The inspection of plates was made visually after every 1 hour of incubation (35°C), for up to 4 hours. Color change from orange to yellow indicated resistance to polymyxin B.

Polymyxin B NP Elution

NP macroelution. The test was performed based on Ngudsuntia *et al.* (2021)[34], with modifications. For each isolate, we used two tubes, containing 14 mL of NP solution in each: 10% anhydrous glucose, cation-adjusted Mueller-Hinton broth (Sigma-Aldrich, USA) and phenol red (Sigma-Aldrich, USA).[20] In one tube, a 300 IU polymyxin B disk (Oxoid, United Kingdom) was added to elute in order to reach a final concentration of 2 µg/mL. The tube was kept at room temperature for 30 minutes for elution. Then, 1 mL of standardized bacterial suspension (5.0McFarland) was added to each tube, to obtain a final bacterial concentration of $\pm 10^8$ CFU/mL.

Results were read by visual inspections after every 1 hour of incubation (35°C), for up to 4 hours. Isolates were considered resistant to polymyxin B when color change (orange to yellow) was evidenced in both tubes (Figure 1).

NP microelution. The miniaturized version, the NP microelution, consisted of two steps, the antibiotic elution, and the test itself. In the elution step, the antibiotic solution was prepared: 2 disks of polymyxin B 300 IU (Oxoid) were added in 15 mL of NP solution, kept at room temperature for 30 minutes, and at 35°C for another 4 hours, in order to complete the antibiotic elution from the disk to the broth, reproducing the full incubation period of PBDE.

For the test step, isolates were evaluated in microtiter plates, where 150 µL of antibiotic-free NP solution was pipetted in one well of the plate, and in another well, 150 µL of NP solution containing the previously eluted antibiotic. Then, 50 µL of a standardized bacterial suspension (3.0 McFarland) was inoculated into each well, reaching a final bacterial concentration of $\pm 10^8$ CFU/mL. After adding the suspension, the well containing the antibiotic had a final concentration of 3 µg/mL of polymyxin B. Plates were incubated at 35°C and read visually every 1 hour for up to 4 hours. Color change of both wells (growth control and test) from orange to yellow indicated resistance to polymyxin B (Figure 2). Whenever color change was unclear, the isolate was considered undetermined.

RESULTS

According the BMD results BMD, 46.4% (39/84) of *Enterobacterales* were resistant to polymyxin B, including 1 isolate of *S. marcescens*, intrinsically resistant, while 53.6% (45/84) were susceptible to polymyxin B. MICs ranged from ≤0.125 µg/mL to >64 µg/mL, with 9.5% (8/84) of isolates presenting borderline MICs (2 or 4 µg/mL), as shown in Table 1.

Compared to BMD, PBDE correctly identified 41 out of 45 isolates susceptible to polymyxin B, with a specificity of 91.1%, positive predictive value (PPV) of 90.7% and major error (ME) of 4.8%. Indeed, it was observed 4 false-positive *K. pneumoniae* (MICs of 0.5 (n=2), 1 (n=1) and 2 (n=1) µg/mL). All 39 resistant isolates were positive in PBDE, presenting 100% sensitivity and negative predictive value (NPV), and no very major errors (VME). Overall, PBDE presented a categorical agreement (CA) of 95.2% among our bacterial population.

Of the 45 isolates susceptible to polymyxins, 43 were negative in RPNP: specificity, PPV and ME were 95.6%, 95.1% and 2.4%, respectively. False-positive results occurred for 2 isolates of *K. pneumoniae* (MICs of 0.25 and 1 µg/mL). We did not observe VME, and sensitivity and NPV were 100%. RPNP identified 97.4% (38/39) of the truly positive (resistant)

isolates within 2h of incubation, with 3h of incubation being necessary only for the *S. marcescens* (MIC = >64 µg/mL).

When NP macroelution was compared to BMD, 100% of sensitivity, 100% of NPV and no VME were observed. However, one false-positive (MIC = 1 µg/mL) and another with an undetermined (considered positive for data analysis) result (MIC = ≤0.125 µg/mL), both for *K. pneumoniae*, were observed: specificity, PPV and ME values of 95.6%, 95.1% and 2.4%, respectively. Among resistant isolates, 92.3% (36/39) expressed positive results within 3h of incubation.

Results of NP microelution were very similar to NP macroelution, with sensitivity and NPV of 100%, and no VME. The methodology presented only 1 false-positive result, for a *K. pneumoniae* (MIC = 1 µg/mL), which was the same isolate presenting a false-positive result in NP macroelution (Table 1): 97.8% specificity, PPV of 97.5% and ME of 1.2%. As with the RPNP methodology, 97.4% (38/39) of true positive results were observed within 2h, except for one *K. pneumoniae* isolate (MIC = 8 µg/mL).

DISCUSSION

The occurrence of polymyxins resistance among carbapenem-resistant Gram-negative bacilli, especially *Enterobacterales*, is increasing in many regions worldwide. In this context, the rapid and accurate detection of this resistance became more necessary, as polymyxins-centered therapeutical schemes are, still, one of the restricted options to adequately treat infections caused by CRE, mainly in countries where ceftazidime-avibactam or other β-lactams and β-lactamase inhibitors combinations are not widely used.[35–39,43]

However, as previously mentioned, this determination is not an easy task for microbiology laboratories.[10,40,41] BMD has recognized disadvantages, highlighting the need for alternative methodologies to determine susceptibility to polymyxins. Among them, CBDE proved to be

simple, easy to adopt in routine, and cheap. Although limited, different studies present satisfactory results,[31–33,42] with CA ranging from 91.18 to 99.5% compared to BMD, VME from 1.1 to 8% and ME from 0 to 12% when *Enterobacterales* were evaluated. In our study, we found a CA of 95.2%, VME of 0% and ME of 4.8%. Interestingly, our study was the only one to reach 100% sensitivity. The reduced number of isolates presenting borderline MICs (n = 8) may justify, at least partially, those results.

Since its first publication in 2016, the RPNP test has been extensively evaluated in several locations, exhibiting sensitivity of 91.0 to 100% and specificity of 70.0 to 100%[21–30], and our results corroborate this good performance (100% sensitivity and 95.6% specificity). It is recognized that certain genera are responsible for drastically influencing the sensitivity and specificity of RPNP, as shown by Simar *et al.* (2017)[44] when evaluating exclusively *Enterobacter* spp., reaching only 25% of sensitivity. Belda-Orlowski *et al.* (2019)[24] observed the influence of this species over test performance as they stratified bacterial population: the specificity of 70% for *Enterobacterales* overall was reduced to 30% when evaluating only *Enterobacter* spp. It is well recognized that heteroresistance to polymyxins, frequently expressed by *Enterobacter* spp. may justify, at least partially, these findings. As our population included only 3 *E. cloacae*, the impact of this species could not be observed. Individually, both PBDE and RPNP presented excellent performances among our bacterial population.

The RCDE, proposed elsewhere[34], assess bacterial populations by exposing them to a colistin concentration of 3.7 µg/ml, after elution from a 10 µg colistin disk into 2.7 ml of NP solution. The inoculum used by the authors was a 1µL loop ($\pm 10^8$ CFU/mL). On the other hand, NP macroelution described here uses 300 IU polymyxin B disks to obtain a concentration of 2 µg/ml (1 disk in 15 mL of NP solution), in order to maintain antibiotic concentration of the methodology approved by CLSI (CBDE). Besides, instead of using 1µL loop, we chose 1 mL

of an adjusted bacterial suspension (5.0 McFarland) as bacterial inoculum, aiming to improve standardization and reproducibility.

In her study, Ngudsuntia *et al.* (2021)[34] found a sensitivity of 94.6%, with a VME of 5.4%, referring to 2 *K. pneumoniae* and 1 *E. cloacae* with false-negative results, all with MIC of 4 µg/mL. Among our population all resistant isolates were correctly identified (100% sensitivity, no VME). On the other hand, the authors found a false-positive result (*K. pneumoniae*, MIC= 2 µg/mL), reaching specificity of 99.4% and ME of 0.6%, which was slightly different from ours (specificity of 95.6% and ME of 2.4%). Our reduced bacterial population may have influenced those findings. The methodologies performed similarly when time needed to identify truly positive results was taken into consideration: 36/39 (92.3%) needed 3h-incubation for NP macroelution and 53/56 (94.6%) for RCDE.

NP macroelution presented higher specificity than PBDE (95.6 *vs* 91.1%) with same sensitivity. Moreover, NP macroelution had the enormous advantage of generating results in a shorter time: up to 4h *vs* 16-20h.

Besides, the miniaturized version of the methodology, the NP microelution performed better, with only one false-resistant isolate (ME of 1.2%) the same isolate that was not correctly evaluated in the NP macroelution. Comparing NP microelution results to those of RPNP, we observed a greater specificity in the newly proposed methodology (97.8 *vs* 95.6%), with no clear reasons for that. Both methodologies were able to provide 97.4% of positive results within 2 hours.

One could mention that a disadvantage of NP microelution would be the need of preparing NP solution with the antibiotic eluted when performing the test. To exclude this step from the methodology, we evaluated the test using a solution previously prepared and stored (4-8°C) for

30 days. The pre-eluted stored and the freshly prepared solution were evaluated at the same time, in the same plate, with a subset of 7 clinical isolates. The results were fully concordant.

Our study has some limitations. The reduced number of bacterial isolates evaluated, mainly those with borderline MICs, strongly recognized by its interference in test accuracies, is probably the most important one.

CONCLUSION

The NP macro and microelution tests proved to be excellent alternatives for determining the susceptibility to polymyxin B when compared to the reference BMD and the original methodologies (RPNP and PBDE). Due to their lower cost, easy execution, and faster release of results, both methodologies can be routinely implemented in clinical laboratories, even with the possibility of store the eluted solution from the antibiotic for long periods. Because of the reduced volumes, NP microelution seems to adapt better to the routine of microbiology laboratories. However, studies evaluating isolates with borderline MICs and a greater number of species, including species recognized by its influence on the test accuracy, such as Enterobacter spp., are still needed.

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TABLES

Table 1: Results of PBDE, RPNP, Macroelution NP and Microelution NP for the detection of susceptibility to polymyxin B among *Enterobacterales*.

Isolates	*MIC[a] Polymyxin B (µg/mL)*	*PBDE*	*RPNP*	*Positivity time (h)*	*Macroelution*	*Positivity time (h)*	*Microelution*	*Positivity time (h)*
K. pneumoniae	>64	P	P	2	P	3	P	2
K. pneumoniae	>64	P	P	2	P	3	P	1
K. pneumoniae	>64	P	P	2	P	3	P	1
K. pneumoniae	64	P	P	2	P	2	P	1
K. pneumoniae	64	P	P	2	P	3	P	2
K. pneumoniae	64	P	P	2	P	3	P	2
K. pneumoniae	64	P	P	2	P	4	P	2
K. pneumoniae	64	P	P	2	P	3	P	2
K. pneumoniae	64	P	P	2	P	3	P	2
K. pneumoniae	64	P	P	2	P	3	P	2
K. pneumoniae	64	P	P	2	P	3	P	1
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	1
K. pneumoniae	32	P	P	2	P	3	P	1
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	1
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	32	P	P	2	P	3	P	2
K. pneumoniae	16	P	P	1	P	3	P	2
K. pneumoniae	16	P	P	2	P	3	P	2
K. pneumoniae	16	P	P	2	P	3	P	2
K. pneumoniae	16	P	P	2	P	3	P	2
K. pneumoniae	16	P	P	2	P	3	P	2
K. pneumoniae	16	P	P	2	P	2	P	2
K. pneumoniae	16	P	P	2	P	3	P	2
K. pneumoniae	16	P	P	2	P	3	P	2
K. pneumoniae	8	P	P	2	P	2	P	4
K. pneumoniae	4	P	P	2	P	4	P	2
K. pneumoniae	4	P	P	2	P	3	P	1
K. pneumoniae	2	P	N	-	N	-	N	-
K. pneumoniae	2	N	N	-	N	-	N	-
K. pneumoniae	2	N	N	-	N	-	N	-
K. pneumoniae	2	N	N	-	N	-	N	-
K. pneumoniae	2	N	N	-	N	-	N	-
K. pneumoniae	1	N	N	-	P	4	P	2
K. pneumoniae	1	N	N	-	N	-	N	-
K. pneumoniae	1	P	N	-	N	-	N	-
K. pneumoniae	1	N	N	-	N	-	N	-
K. pneumoniae	1	N	P	2	N	-	N	-
K. pneumoniae	1	N	N	-	N	-	N	-

K. pneumoniae	1	N	N	-	N	-	N	-
K. pneumoniae	0.5	N	N	-	N	-	N	-
K. pneumoniae	0.5	N	N	-	N	-	N	-
K. pneumoniae	0.5	N	N	-	N	-	N	-
K. pneumoniae	0.5	P	N	-	N	-	N	-
K. pneumoniae	0.5	N	N	-	N	-	N	-
K. pneumoniae	0.5	P	N	-	N	-	N	-
K. pneumoniae	0.5	N	N	-	N	-	N	-
K. pneumoniae	0.5	N	N	-	N	-	N	-
K. pneumoniae	0.5	N	N	-	N	-	N	-
K. pneumoniae	0.5	N	N	-	N	-	N	-
K. pneumoniae	0.25	N	N	-	N	-	N	-
K. pneumoniae	0.25	N	N	-	N	-	N	-
K. pneumoniae	0.25	N	N	-	N	-	N	-
K. pneumoniae	0.25	N	N	-	N	-	N	-
K. pneumoniae	0.25	N	P	3	N	-	N	-
K. pneumoniae	0.25	N	N	-	N	-	N	-
K. pneumoniae	0.25	N	N	-	N	-	N	-
K. pneumoniae	0.25	N	N	-	N	-	N	-
K. pneumoniae	0.25	N	N	-	N	-	N	-
K. pneumoniae	≤0.125	N	N	-	I	-	N	-
K. pneumoniae	≤0.125	N	N	-	N	-	N	-
K. pneumoniae	≤0.125	N	N	-	N	-	N	-
K. pneumoniae	≤0.125	N	N	-	N	-	N	-
K. pneumoniae	≤0.125	N	N	-	N	-	N	-
K. oxytoca	2	N	N	-	N	-	N	-
K. oxytoca	≤0.125	N	N	-	N	-	N	-
K. oxytoca	≤0.125	N	N	-	N	-	N	-
E. cloacae	0.5	N	N	-	N	-	N	-
E. cloacae	0.25	N	N	-	N	-	N	-
E. cloacae	≤0.125	N	N	-	N	-	N	-
E. coli	0.5	N	N	-	N	-	N	-
E. coli	0.5	N	N	-	N	-	N	-
E. coli	≤0.125	N	N	-	N	-	N	-
S. marcescens	>64	P	P	3	P	4	P	2
E. coli ATCC 25922[b]	0,5	N	N	-	N	-	N	-
M. morganii[c]	>64	P	P	2	P	3	P	2

[a] MIC determined by BMD; [b] Negative control; [c] Positive control.

MIC, minimum inhibitory concentration; PBDE, polymyxin B broth disk elution; RPNP, rapid polymyxin NP test; P, positive growth (resistant); N, negative growth (susceptible); I, indefinite growth.

Table 2: Performance of methods among *Enterobacterales* compared to broth microdilution.

Parameter	*PBDE*	*RPNP*	*Macroelution NP*	*Microelution NP*
CA	95.2%	97.6%	97.6%	98.8%
Sensitivity	100.0%	100.0%	100.0%	100.0%
Specificity	91.1%	95.6%	95.6%	97.8%
PPV	90.7%	95.1%	95.1%	97.5%
NPV	100.0%	100.0%	100.0%	100.0%
ME	4.8%	2.4%	2.4%	1.2%
VME	0.0%	0.0%	0.0%	0.0%

PBDE, polymyxin B broth disk elution; RPNP, rapid polymyxin NP test; CA, categorical agreement; PPV, positive predictive value; NPV, negative predictive value; ME, major error; VME, very major error.

FIGURES LEGENDS

Figure 1: Representative results of the NP macroelution test at every hour of reading, for up to 4h, with growth being evidenced from color change (orange to yellow). For each image of two tubes, the tube on the right contains the eluted polymyxin B disk, reaching a concentration of 2 µg/mL. A: Susceptible isolate due to permanence of orange color in tube containing antibiotic disk. B: Resistant isolate due to color change of tube containing antibiotic disk.

Figure 2: NP microelution test results at each hour of reading for up to 4h. The color change of the wells from orange to yellow indicates bacterial growth. A: Antibiotic-free column of wells (growth control). B: Column of wells with NP solution where antibiotic disks were previously eluted, resulting in a final concentration of 3 µg/mL of polymyxin B. C: Resistant isolate due to color change in the well containing the antibiotic. D: Sensitive isolate due to continuity of orange staining of antibiotic-containing well.

FIGURES

Figure 1:

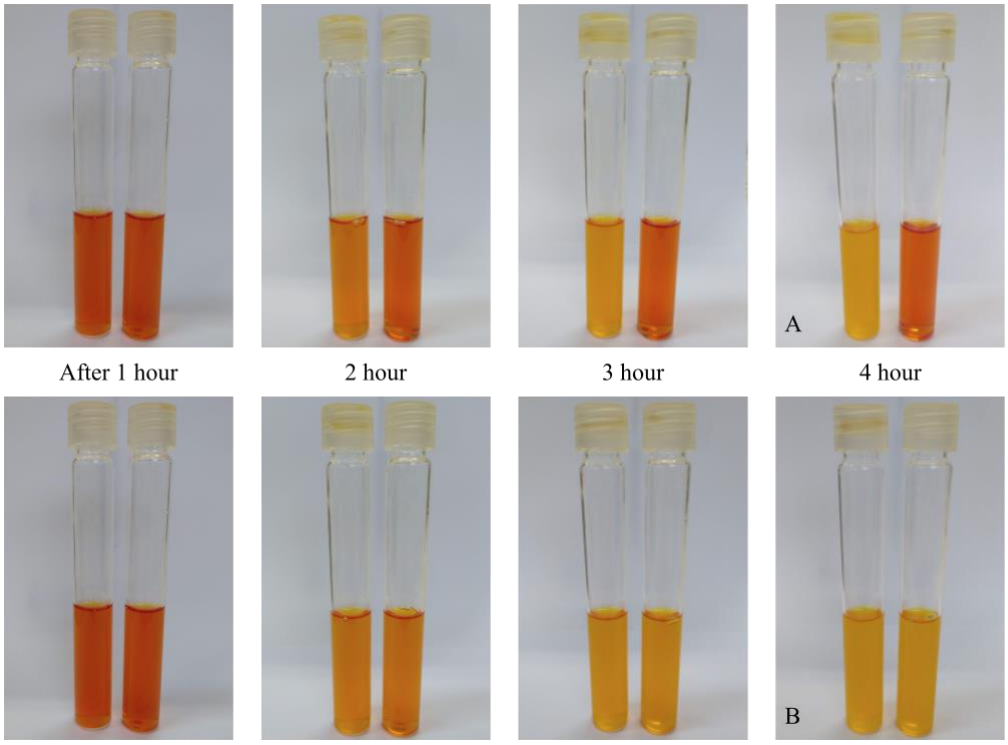
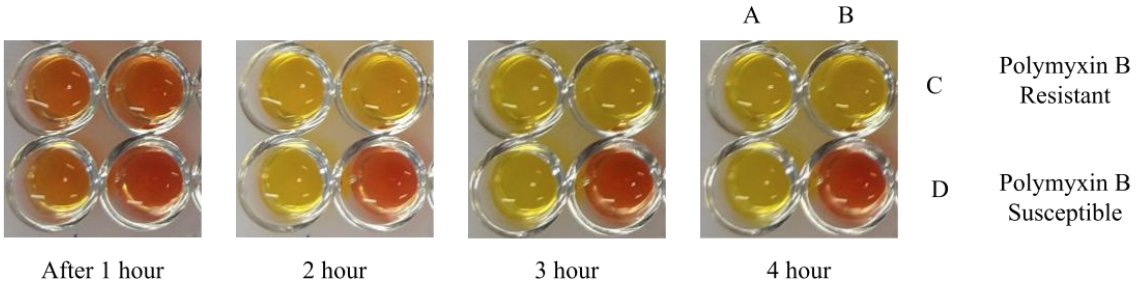


Figure 2:


A
B
C
Polymyxin B Resistant
D
Polymyxin B Susceptible
After 1 hour
2 hour
3 hour
4 hour

ANEXO 1

Manuscript Submission Guidelines and Policies for *Microbial Drug Resistance*

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Book:

Hauser AR, Rello J, eds. Severe Infections Caused by Pseudomonas aeruginosa. Boston, MA: Kluwer Academic Publishers; 2003.

Chapter in a book:

Jarvis WR. Epidemiology and control of?Pseudomonas aeruginosa?infections in the intensive care unit. In: Hauser AR, Rello J, eds. Severe Infections Caused by?Pseudomonas aeruginosa. Boston, MA: Kluwer Academic Publishers; 2003, pp. 153–168.

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Website:

Centers for Disease Control and Prevention. National Antimicrobial Resistance Monitoring System (NARMS) Now: Human Data. U.S. Department of Health and Human Services, CDC, Atlanta, GA. 2019. Available at https://cdc.gov/narmsnow

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