https://doi.org/10.52418/moldovan-med-j.66-1.23.02
UDC: 615.281.9.012:547.995.12

Bactericide polymers obtained from nitrofuran and chitosan derivatives

[1]Viorel Prisacari, [1]Diana Buga, [2]Roman Rusnac, [2]Liliana Caisim, [2]Stefan Robu

[1]Department of Epidemiology, *Nicolae Testemitanu* State University of Medicine and Pharmacy
[2]Department of Chemistry, State University of Moldova, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contribution are available at the end of the article

*Corresponding author – Viorel Prisacari, e-mail: viorel.prisacari@usmf.md

Manuscript received December 19, 2022; revised manuscript February 27, 2023; published online March 10, 2023

Abstract

Background: In this study, the synthesis and characterization of chitosan polymeric materials grafted with nitrofuran derivatives – furaceline and isofural in terms of antibacterial properties and prolongation of their action were performed.
Material and methods: Synthesis of chitosan analog polymers with maleic anhydride was performed, followed by grafting of nitrofuran derivatives using ethyl chloroformate. The chemical composition of the obtained polymers was confirmed by FT-IR spectroscopy. Antibacterial study has been performed on a wide range of Gram-positive and Gram-negative microorganisms.
Results: Chitosan derivatives with a content of 30 mol% of maleic anhydride were obtained. To the analogous polymer "chitosan maleinized" the medicinal products isofural or furaciline with the help of the ethyl chloroformate were functionalized. By comparative analysis of the IR spectra of the final products with the IR spectra of maleinized chitosan and furacilin or isofuran was demonstrated the individual structure of the polymeric preparations "maleinized chitosan grafted with furacilin" / "maleinized chitosan grafted with isofural". The antibacterial substances, isofural and furaciline, among nitrofurans, being grafted with chitosan maleinized, keep their bactericidal activity in the limits of 75-300 μg/mL. The polymeric materials from chitosan maleate grafted with isofural or furacillin in a ratio of 70:30 have a prolonged antibacterial action (observation period 72 hours).
Conclusions: It has been found that isofural and furacilin, among nitrofurans, being grafted on chitosan polymeric material, retain their bactericidal activity and possess prolonged antibacterial action.
Key words: chitosan, furaciline, isofural, grafted copolymers, Gram-positive and Gram-negative microorganisms.

Cite this article
Prisacari V, Buga B, Rusnac R, Caisim L, Robu S. Bactericide polymers obtained from nitrofuran and chitosan derivatives. *Mold Med J*. 2023;66(1):13-17. https://doi.org/10.52418/moldovan-med-j.66-1.23.02.

Introduction

Starting from the years 75-80 of the last century, the chemical-pharmaceutical direction for the elaboration of antimicrobial polymeric materials began to develop rapidly [1-3]. Pharmaceutical chemists were the first to believe that the future in in

creasing the effectiveness of antibiotics and other antibacterial preparations will return to their conjugation with polymeric materials and thus have a higher antimicrobial potential, with tens of times longer activity and reduced toxicity [3].

A special role in the synthesis of antimicrobial preparations on polymeric support have the researches that demonstrated the possibility of grafting of a series of antimicrobial drugs with dextran, polyvinyl alcohol, polyethylene glycol (PEG) and PEG derivatives [4]. Antibacterial preparations conjugated with polymeric materials possess relatively better antimicrobial activity.

It is known from the literature that in the years 1980-1990 were chemically coupled by covalent bonds the preparations with action on the nervous system, such as adrenaline, isoproternol and others with the synthetic copolymer of N-(3-oxypropyl) glutamine and N-aminophenylalanine. The polymeric derivatives obtained showed activity in the initial catecholamines [5, 6].

In the literature are also described some polymers, such as the copolymer of N-vinylpyrrolidone with crotonic aldehyde, used for the grafting of antibacterial preparations [7, 8]. The authors showed that these compounds have a prolonged activity within 10-12 days, unlike the usual preparations that have activity for 1-2 days [8]. The use of polymeric preparations led to a reduction of the dose by 2-3 times, compared to the initial preparations.

In some publications there are described processes for grafting antibiotics from the penicillin group to synthetic copolymers, such as N-vinylpyrrolidone with crotonic or acrylic acid and from the styrene-butadiene block copolymer. The last preparation is also recommended for obtaining layers and films with antibacterial effect [9].

From the nitrofuran derivatives, a special role is played by N'-[(5-nitrofuran-2-yl) methylidene]pyridine-4-carbohydrazide, also called isofural [10]. This homologous antibacterial preparation, close to the chemical structure with furacillin but with more advanced bactericidal properties, was obtained by the classical condensation reaction of pyr-

idine-4-carbohydrazide with 5-nitrofuran-2-carbaldehyde [11]. The authors showed that the activity of this preparation is superior to that of other preparations in the nitrofuran group.

The aim of this paper is to investigate the possibility of grafting furacillin and isofural to the natural chitosan polymer, to confirm the chemical structure of the polymer-analogs obtained with the help of IR spectroscopy and to evaluate the antibacterial properties.

Material and methods

In this paper, the synthesis and research of polymeric materials with bactericidal properties from chitosan grafted with nitrofuran derivatives were performed.

Synthesis of polymers was carried out in two stages: in stage 1 the synthesis of the polymer-chitosan analog functionalized with maleic anhydride (MA) represented in fig. 1 was performed. The MA content was from 30 to 50 mol%.

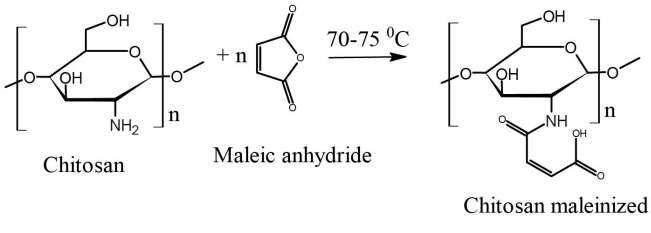

Fig. 1. Scheme of functionalization response of maleic anhydride to chitosan

Grafting was carried out in a solution of N,N-dimethylformamide (DMF) with a chitosan concentration of 1.0% at 70-75 °C for 3 hours. The end of the reaction was confirmed by the thin layer chromatography. The reaction yield is about 80%.

In the second stage, the functionalization of isofural and furacilin in maleinized chitosan (step I) was performed. Synthesis was performed according to the scheme shown in fig. 2 (step II).

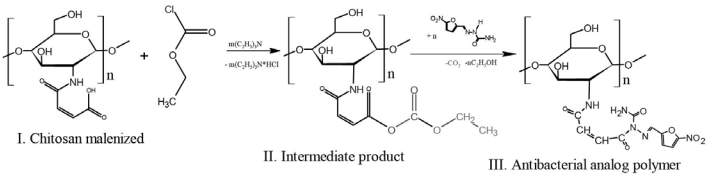

Fig. 2. Reaction schedule for functionalization of furacilin/isofural in chitosan maleinized

Similarly, the isofural to maleinized chitosan was functionalized as follows: to the solution containing 1.0 g of maleinized chitosan dissolved in 50 mL DMF, at the temperature of 3-5 °C, with dropwise stirring were added 0.8 mL (7.8 mmol) of triethylamine and after 10 minutes 0.7 mL of ethyl chloroformate. After stirring for 30 minutes at 3-5 °C to the intermediate product (II) the solution of isofural in DMF (0.6 g dissolved in 10 mL DMF) was added. The resulting reaction product was stirred for another 3-4 hours at room temperature, then after concentrating the solution by vacuum distillation of the polymer the final polymer analog (III) was separated by sedimentation in hexane, then in diethyl ether.

The chemical structure of chitosan maleinized and other analogous polymers was investigated using IR spectroscopy. The BRUKER ALPHA platinum ATR spectrometer was used for the research (fig. 3).

The method for evaluating antibacterial properties

Evaluation of antibacterial properties of the synthetic origin substances (maleinized chitosan with isofural (70:30 mol%) and chitosan maleinized with furacilin (70:30 mol%) was realized applying the method of serial dilutions in liquid. As a nutrient medium 2% meat – peptone broth was used. As reference cultures were used Gram-positive and Gram-negative microorganisms (*Staphylococcus aureus* (t.

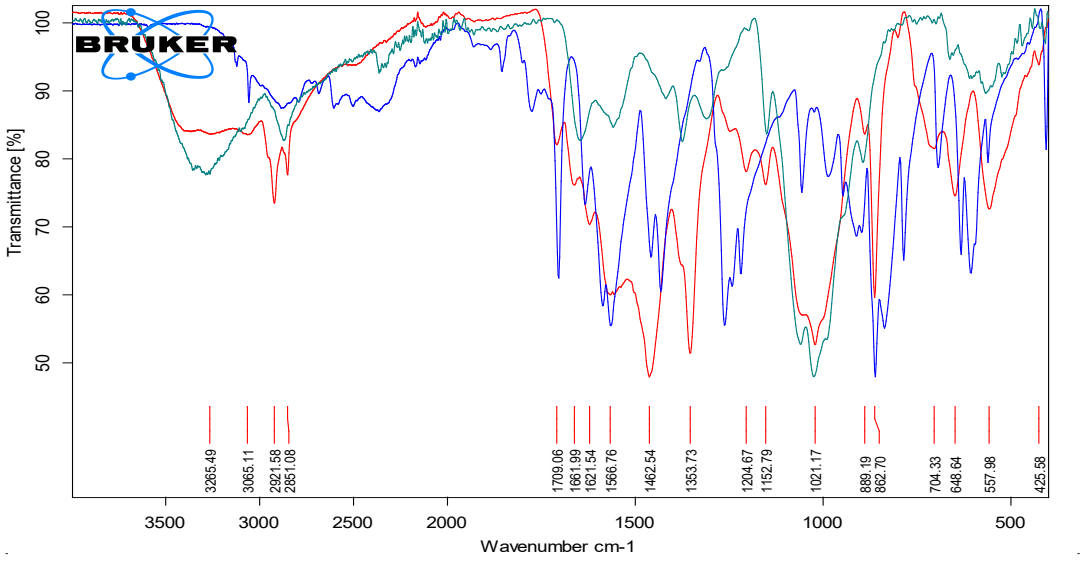

Fig. 3. FT – IR spectrum of chitosan maleinized compared to chitosan and maleic anhydride

209), methicillin-resistant *Staphylococcus aureus* (MRSA) (t. NCTC 12493), *Enterococcus faecalis* (t. ATCC 19433), *Escherichia coli* (t. ATCC 25922), *Klebsiella pneumoniae* (t. 3534/51), *Proteus mirabilis* (t. ATCC 3177), *Acinetobacter baumanii* (t. ATCC 19606)).

Bacteriostatic activity was assessed in the absence of growth of microorganisms in the liquid nutrient medium. The bactericidal activity was evaluated based on the lack of growth of microorganisms on the solid nutrient medium – meat peptone agar, after the repeated sowing and ulterior thermostating during 24, 48 and 72 hours.

Results and discussion

1. Spectral analysis of the polymer-analogs synthesized

The IR spectra of chitosan (1), maleic anhydride (2) and chitosan maleinized (3) are presented in fig. 3. Figure shows in the spectrum of chitosan maleate the presence of new vibrations that are missing in the spectrum of chitosan and in the spectrum of maleic anhydride. For example, vibrations ν = 3065 cm^{-1} characteristic of the amide groups -NH-CO-. From the spectrum of malignant chitosan, new vibrations are also observed at ν = 1566-1709 cm^{-1} characteristic of carbonyl groups. From the spectra there is also observed a massive increase in vibrations ν = 1353 cm^{-1} characteristic of carbonate groups. In the spectrum of chitosan maleate we also notice the appearance of a massive band at ν – 862 cm^{-1} characteristic of the double bonds in maleic anhydride grafted to chitosan macromolecules.

Fig. 4 shows the IR spectrum of the final chitosan maleinized polymer functionalized with furacillin, and the furacillin as a control sample.

Figure 5 shows the increase of the absorption bands 1540-1590 cm^{-1} characteristic of the amino (secondary) groups as well as the appearance of the new vibrations 3200-3300 cm^{-1} characteristic of the secondary and tertiary amide groups. The same changes were observed in the case of chitosan maleinized-isofural.

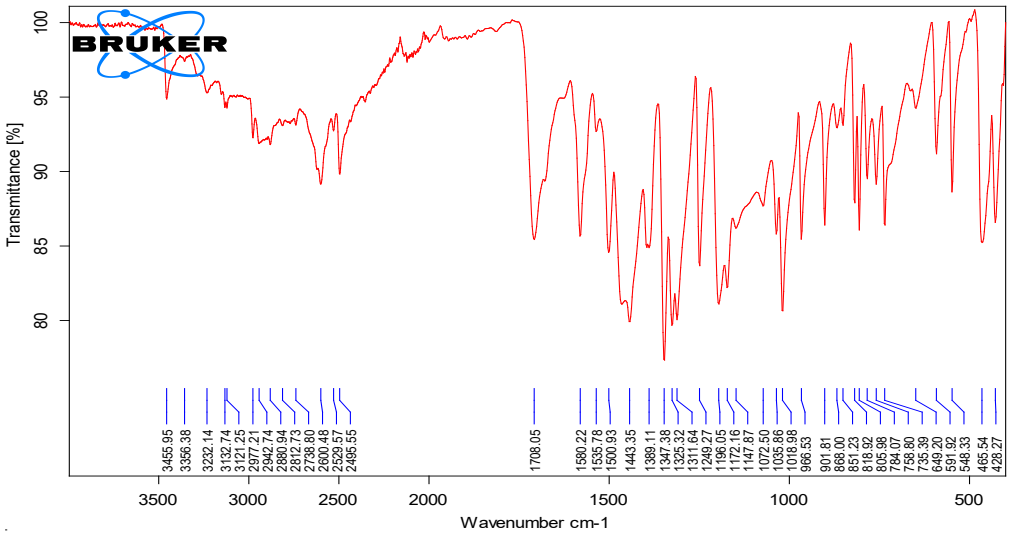

Fig. 4. IR spectrum of furacillin and furacillin grafted to chitosan maleinized (70-30 mol%)

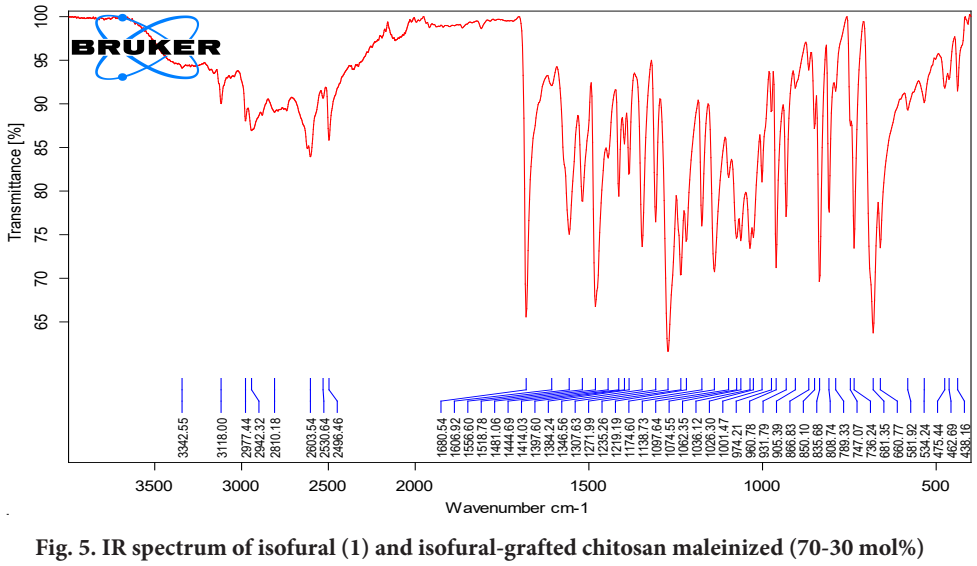

Fig. 5. IR spectrum of isofural (1) and isofural-grafted chitosan maleinized (70-30 mol%)

2. Evaluation of antibacterial action

As a result of the study of the antibacterial properties of the researched substances, it was found that both substances obtained from chitosan grafted with isofural or furacillin, possess antibacterial activity in a wide range of microorganisms, Gram-positive and Gram-negative (tab. 1 and 2).

The bactericidal activity of chitosan maleinized – isofural (70:30), after 24 hours of incubation on peptone agar, varies within the concentrations of 150 – ≥300 mcg/mL and constitutes for *S. aureus* (t.209) – 150 mcg/ml, and for the other species of gram-positive and gram-negative microorganisms – ≥300 mcg/mL.

The bactericidal activity of chitosan maleinized – furacilin (70-30), after 24 hours of incubation on peptone agar, varies within the limits of concentrations 150 – ≥300 mcg / mL and constitutes for *E. coli* – 150 mcg/mL, and for all other species of microorganisms – ≥300 mcg / mL.

As a result of the long incubation, 48 and 72 hours, of the cultures of microorganisms sown on peptonate broth, there have been obvious changes in the bactericidal activity of both substances over time.

The bactericidal activity of chitosan maleinized – isofural (70:30) in the condition of prolonged exposure increased for methicillin-resistant *Staphylococcus aureus* with 4 dilutions, from the concentration of the substance of 300 mcg/mL to 18.75 mcg/mL, for *S. aureus* (t.209) – with two di-

Table 1. Bactericidal activity of the polymer of the analogue "Chitosan maleinized – isofural (70:30)" (minimum bactericidal concentration (μg / mL))

Test – Gram-positive bacterial cultures									
Dose (mcg/ml)	*S.aureus (t.209)*			*MRSA (t.NCTC 12493)*			*E.faecalis (t.ATCC 19433)*		
	24	48	72	24	48	72	24	48	72
300	-	-	-	-	-	-	±	-	-
150	-	-	-	±	-	-	±	+	+
75	±	-	-	±	-	-	+	+	+
37.5	±	-	-	±	-	-	+	+	+
18.75	+	+	+	±	-	-	+	+	+
9.37	+	+	+	+	+	+	+	+	+
Test – Gram-negative bacterial cultures									
Dose (mcg/ml)	*E.coli (t.ATCC 25922)*			*K.pneumoniae (t.3534/51)*			*P.mirabilis (t.ATCC 3177)*			*A.baumannii (t.ATCC 19606)*		
	24	48	72	24	48	72	24	48	72	24	48	72
300	±	-	-	+	±	±	+	-	-	+	-	-
150	+	±	+	+	+	+	+	+	+	+	+	+
75	+	+	+	+	+	+	+	+	+	+	+	+
37.5	+	+	+	+	+	+	+	+	+	+	+	+
18.75	+	+	+	+	+	+	+	+	+	+	+	+

Table 2. Bactericidal activity of the polymer of the analogue "Chitosan maleinized – furacilin (70-30)"

Test – Gram-positive bacterial cultures									
Dose (mcg/ml)	*S.aureus (t.209)*			*MRSA (t.NCTC 12493)*			*E.faecalis (t.ATCC 19433)*		
	24	48	72	24	48	72	24	48	72
300	±	-	-	±	-	-	-	-	-
150	±	-	-	±	-	-	±	+	+
75	±	-	-	±	+	+	±	+	+
37.5	±	±	±	±	±	+	+	+	+
18.75	±	±	+	+	±	+	+	+	+
Test – Gram-negative bacterial cultures									
Dose (mcg/ml)	*E.coli (t.ATCC 25922)*			*K.pneumoniae (t.3534/51)*			*P.mirabilis (t.ATCC 3177)*			*A. baumannii (t.ATCC 19606)*		
	24	48	72	24	48	72	24	48	72	24	48	72
300	-	-	-	±	-	-	+	+	+	-	-	-
150	-	-	-	±	-	-	+	+	+	+	-	-
75	+	±	-	+	+	+	+	+	+	+	+	+
37.5	+	+	+	+	+	+	+	+	+	+	+	+
18.75	±	±	+	+	±	+	+	+	+	+	+	+

lutions, for *E. faecalis, E. coli, P. mirabilis* and *A. baumanii* – with one dilution, and for *K. pneumoniae* remained unchanged (Table 1).

The bactericidal activity of chitosan maleinized – furacilin (70-30) in the condition of prolonged exposure (72 hours) increased for *S. aureus* (t.209) by three dilutions, for methicillin-resistant *S. aureus* and *K. pneumoniae* – with two dilutions, for *E. coli* and *A. baumanii* – with one dilution, for *E. faecalis* and *P. mirabilis* remained unchanged (tab. 2).

Conclusions

1. Chitosan derivatives with a content of 30 mol% of maleic anhydride were obtained. To the analogous polymer "chitosan maleinized" the medicinal products isofural or furacilin with the help of the ethyl chloroformate were functionalized.

2. By comparative analysis of the IR spectra of the final products with the IR spectra of maleinized chitosan and furacilin or isofuran was demonstrated the individual structure of the polymeric preparations "maleinized chitosan grafted with furacilin" / "maleinized chitosan grafted with isofural".

3. The antibacterial substances, isofural and furacilin, among nitrofurans, being grafted with chitosan maleinized, keep their bactericidal activity in the limits of 75-300 μg / mL.

4. The polymeric materials from chitosan maleate grafted with isofural or furacilin in a ratio of 70:30 have a prolonged antibacterial action (observation period 72 hours).

References

1. Jenkins M, editor. Biomedical polymers. Boca Raton: CRC Press; 2007. 224 p.
2. Simionescu CI, Gorduza V. Polimeri biocompatibili şi biologic activi [Biocompatible and biologically active polymers]. Bucharest: Editura Academiei Republicii Socialiste Romania; 1980. 479 p. Romanian.
3. Plate NA, Vasil'ev AE. Fiziologicheski aktivnye polimery [Physiologically active polymers]. Moscow: Khimiia; 1986, 296 p. Russian.
4. Venter JC. Immobilized and insolubilized drugs, hormones, and neurotransmitters: properties, mechanisms of action and applications. Pharmacol Rev. 1982;34(2):153-87.
5. Goodman M, Verlander MS, Kaplan NO. Chemical and biological aspects of peptide catecholamine conjugates. J Macromol Sci. Part A: Chemistry. 1979;13(4):529-43. https://doi.org/10.1080/00222337908066611.
6. Solovsky MV, Ulbrich K, Kopecek J. Synthesis of N-(2-hydroxypropyl)-methacrylamide copolymers with antimicrobial activity. Biomaterials. 1983;4(1):44-8. https://doi.org/10.1016/0142-9612(83)90069-8.
7. Groves ES, Aldwin L, Winkelhake JL, Nitecki DE, Gauney S, Rudolph AA, inventors. Polymer/antibiotic conjugate. Patent WO/1990/015628.
8. Peters JT,Wechsler M, Peppas NA. Advanced biomedical hydrogels: molecular architecture and its impact on medical applications. Regen Biomater. 2021;8(6):rbab060. doi: 10.1093/rb/rbab060.
9. Ghioca P, Robu S, Prisacari V, Filip V, Spurcaciu B, Iancu L, Grigorescu RM. Styrene-butadiene block-copolymers used as immobilization support for drugs with prolonged antibiotic effect. Mater Plast. 2014;51(1):94-96.
10. Prisacari V, Buraciov S, Dizdari A, Stoleicov S, Tapcov V. [Izonicotinoil-hidrazone aldehyde 5-nitro-2-furan –a new organic compound with antibacterial activity]. In: [Scientific Annals of *Nicolae Testemitanu* State University of Medicine and Pharmacy]. Chisinau: Medicina; 2002. p. 255-259. Romanian.
11. Prisacari V. Synthetic substances – antibacterial and antifungal potential products. In: 2nd International Conference on Nanotechnologies and Biomedical Engineering; 2013 April 18-20; Chisinau, Republic of Moldova: Proceedings. Chisinau; 2013. P. 385-389. [cited 2022 Oct 21]. Available from: http://repository.utm.md/bitstream/handle/5014/5374/Conf_2_ICNBME_2013_p385_389.pdf?sequence=1&isAllowed=y.

Authors' ORCID iDs and academic degrees

Viorel Prisacari, MD, PhD, Professor – https://orcid.org/0000-0002-8694-2327
Diana Buga, MD, PhD, Scientific Researcher – https://orcid org/0000-0002-4733-9592
Roman Rusnac, MD, PhD, Associate Professor – https://orcid org/0000-0002-5713-5251
Liliana Caisim, MD, Master Student – https://orcid.org/0000-0003-1147-3033
Stefan Robu, MD, PhD, Associate Professor – https://orcid.org/0000-0002-9804-5543

Authors' contributions

VP designed the research; DB did statistics; RR, LC interpreted the data; SR drafted the manuscript. All the authors revised the manuscript critically and approved the final version of the manuscript.

Funding

This study was supported by the grant from the National Agency for Research and Development of the Republic of Moldova (project No 20.80009.8007.05). The authors take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate

The research was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, protocol No 46 of 12.04.2018.

Conflict of interests. No competing interests were disclosed.