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EXAMINATION OF ANTIMICROBIAL ACTIVITY OF SELECTED NON-ANTIBIOTIC MEDICINAL PREPARATIONS

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Abstract: The aim of this study was to detect and characterize the antimicrobial activity of non-antibiotic drugs, selected from the pharmaceutical products analyzed during the state control performed in National Medicines Institute, Warszawa, Poland. In 2010, over 90 pharmaceutical preparations have been randomly chosen from different groups of drugs. The surveillance study was performed on standard ATCC microbial strains used for drug control: *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*. It was shown that the drugs listed below inhibited growth of at least one of the examined strains: Arketis 20 mg tab. (paroxetine), Buvasodil 150 mg tab. (buflomedile), Halidor 100 mg tab. (bencyclane), Hydroxyzinum espefa 25 mg tab. (hydroxyzine), Norifaz 35 mg tab. (risedronate), Strattera 60 mg cap. (atomoxetine), Tamiflu 75 mg tab. (oseltamivir), Valpro-ratiopharm Chrono 300 mg tab. with longer dissolution (valproate), Vetminth oral paste 24 g+3 g/100 mL (niclozamide, oxybendazol). Strattera cap. showed broad activity spectrum. It inhibited growth of all examined strains (MIC of active substance – atomoxetine ranged between 2.6–13 mg/mL).

Keywords: non-antibiotics, drugs, antimicrobial activity, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans*

Great variety of compounds involved in diseases treatment of non-infectious etiology have shown some antimicrobial activity *in vitro*, against bacteria and other microorganisms (1–3). Such compounds are called "non-antibiotics". By the end of the nineteenth century, the dyes were known to possess antimicrobial activity, for instance, Paul Ehrlich used methylene blue (one of phenothiazines compounds) as an antimicrobial agent (4). So far, a lot of attention has been focused on phenothiazines, thioxanthenes and other agents with affinity to cellular transport systems influencing the structure of cellular membrane or ions transport etc. (5, 6).

Antimicrobial activity was found among some "non-antibiotics" present at Polish pharmaceutical market, during several years of drug control in the National Medicines Institute. So far, about 950 drugs randomly chosen from different groups of pharmaceutical products have been examined. During the study (7–13), it was indicated that some of the preparations inhibited growth of at least one of the four examined standard microbial strains. The drugs with the following active substances showed significant antimicrobial activity: amlodipine, acepromazine, butorphanol, cisapride, cisplatin, clomipramine, diltiazem, emadastine, fluvastatin, ketamine, levocabastine, matipranalol, methotrexate, nicergoline, perphenazine, proxymetacaine, sertraline, tegaserole, tetrahydrozoline, ticlopidine and tropicamide.

The aim of this study was the continuation of research and characterisation of the antimicrobial activity of non-antibiotic drugs, obtained during a routine state control performed during the last year in the National Medicines Institute.

EXPERIMENTAL

Materials

The following microorganisms: *Escherichia coli* ATCC 8739*, Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538P and *Candida albicans* ATCC 10231 were used in the study.

The following pharmaceutical products available on the Polish market were randomly chosen for

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the analysis: Alax 35 mg + 42 mg tab. (*aloe capensis folia* , *Frangulae cortex extr. sicc*.), Altacet 1 g tab. (acetate-tartrate Al, boric acid), Alusal 500 mg tab. ($Al(OH)_3$), Amertil 1 mg/mL oral sol. (cetirizini 2 HCl), AnastroLEK 1 mg tab. (anastrozole), Antisedan inj. 5 mg/mL (atipamezole HCl), Apo-Risperid 1 mg/1 mL oral sol. (risperidone), Arketis 20 mg tab. (paroxetine), Aulin 100 mg tab. (nimesulide), Bellapan 0.25 mg tab. (atropine sulfate), Betahistine PLIVA 24 mg tab. (betahistini 2 HCl), Betasol oral sol. (90.0% extr. from*: Pericarpium phaseoli, Radix bardanae, Radix ononidis, Radix rhei, Herba equiseti, Herba fagopyri, Fructus sambuci, Folium betulae,* 10.0% glycerin), Bisopromerck 10 mg tab. (bisoprole fumarate), Bupivacaine WZF Spinal 0,5% Heavy inj. (bupivacaini HCl), Buvasodil 150 mg tab. (buflomedile HCl), Catalin 0.75 mg tab. + solvent for Ele drops (pirenoxine), Cepan cream (extracts of: *Alii cepae* 20 g*; Chamomillae* 5 g*;* heparin Na 5000 UI*,* allantoin 1 g, in 100 g), Cinnarizinum 25 mg tab. (cinnarizine), Coaxil 12.5 mg tab. (tianeptine Na), Co-Diovan 320 mg + 12.5 mg; 320 mg + 25 mg tab. (valsartan, hydrochlorothiazide), Convulex 50 mg/mL syrup (valproate Na), Dehinel Plus (150 + 144 + 50) mg (febantel, pyrantel emboniane, praziquantel), Depogestron 50 mg/mL inj. susp., Diovan 320 mg tab. (valsartan), Diovan 40 mg tab. (valsartan), Donesyn 10 mg tab. (donepezil HCl), Echinacea-ratiopharm 8 mg tab. (*Echinaceae angustifoliae radicis extr. sicc*.), Epitoram 200 mg tab. (topiramate), Etopiryna tab. 300 mg + 100 mg + 50 mg (acetylsalicylic acid, ethenzamide, coffeine), Fenardin 267 mg cap. (fenofibrate), Gabapentin TEVA 800 mg tab. (gabapentin), Gastal 450 mg + 300 mg tab. ($Al(OH)_3$, $MgCO_3$), Gastrolit oral powder (*Chamomilla recutita extr.,* NaCl, $NaHCO_3$, glucose, KCl), Sedazin 20 mg/mL inj. (xylazine), Gopten 0.5 mg tab. (trandolapril), Halidor 100 mg tab. (bencyclane fumarate), Haloperidol 5 mg tab. (haloperidol), Hydroxyzinum espefa 25 mg tab. (hydroxyzine HCl), Inhalol sol. for steam inhalation *(aetheroleum cum: Pini silvestris* 0.1 g/g*; Thymi sp* 0.1 g/g*; Menthae piperitae* 0.2 g/g*; Terebinthinae sp* 0.6 g/g*)*, Iomeron 612.4/mL inj. (lomeprole), Kaldyum 600 mg cap. (KCl), Kalipoz prolongatum 391 mg K^+, tab. longer dissolution (KCl), Kalium Effervescens 782 mg K^+/5 g granulate (KCl + $KHCO_3$), Kelicardina sol. (*Convallariae extr.* 50 g*, Crategi inflorescentiae extr.* 42 g*, Troxerutinum* 0.5 g*, ethanol* 96% 7.5 g*)*, Lafactin 150 mg cap. (venlafaxine), Logista 25 mg tab. (losartan K), Lozap HCT 50 mg + 12.5 mg tab. (losartan K + hydrochlorothiazide), Lumbolin ointment (heparinoids – 100 UI, ethylene-glycol ester monosalicylic acid – 0.1 g; nicotinic acid benzyl ester 0.025 g), MAGNE B6 470 mg + 5 mg tab. (lactate Mg, B6), Marcaine Spinal 0.5% Heavy 5 mg/mL (bupivacaine HCl), Ointment majerankowa *(Majorae herbae extr.)*, MaxFlu (500 +30 + 60) mg tab. (paracetamole, phenylephrine HCl, ascorbic acid), Medazepam 10 mg cap. (medazepam), Mestinon 60 mg tab. (pyridostigmine Br), Metocard 100 mg tab. (methoprolol), Milocardin (300 mg + 300) mg/15g oral drops (ethyl bromoisovalerate, phenobarbital Na), Mirtazapine TEVA 45 mg tab. (mirtazapine), Nebinad 5 mg tab. (nebivolol), Norifaz 35 mg tab. (risedronate Na), Omnipaque 350 mg/mL inj. (iohexole), Orgametril 5 mg tab. (linestrenole), Procto-hemolan 50 mg + 20 mg/g cream (tribenoside, lidocaine HCl), Proscillaridin 250 µg tab. (proscillaridine), Saridon 250 mg + 150 mg + 50 mg tab. (paracetamol, propyphenazone, coffeine), Sputolysin 2 mg/g oral powder (dembrexin HCl), Stomasol oral sol. (in 1 mL tincture of: *Gallae sp* – 304 mg*; Arnicae sp*. – 304 mg*; Tormentillae sp*. – 304 mg*,* 60–70% ethanol), Stopress 8 mg tab. (perindopril tert-butylamine), Strattera 40 mg, 60 mg cap. (atomoxetine), Succus Farfarae oral sol. (*Farfarae folium succus* in 35% ethanol), Symepezil 10 mg tab. (donepezil HCl), Symlozin SR 0,4 mg cap. (tamsulosine HCl), Syndi-35 2 mg + 0.035 mg tab. (cyproterone acetate, ethinylestradiole), Tadenan 50 mg cap. (*Pygei africani cortex ext*.), Tamiflu 75 mg tab. (oseltamivir), Taninal 500 mg tab. (*Tanninum albuminatum*), Theraflu 650 mg + 10 mg oral powder (paracetamole + phenylephrine HCl), Tialorid 5 mg + 50 mg tab. (amiloride HCl, hydrochlorothiazide), Tinctura Ginkgo Bilobae 935 mg/mL oral sol. (*Ginkgo bilobae folii tinct*.), Urogram oral granulate (*Solidaginis herb*. – 20 g*, Levistici rad*. – 13 g*, Cichorr rad*. – 10 g*, Calami rhiz*. – 10 g*, Equiseti herb*. – 7 g*, Betulae fol*. –7 g), Valpro-ratiopharm Chrono 300 mg tab. with longer dissolution (valproate Na + valproic acid), Valzek 80 mg, 160 mg tabl. (valsartan), Vegantalgin H 500 mg + 10 mg tab. (paracetamol + hioscine butylbromide), Vermox 100 mg tab. (mebendazol), Vertigen 16 mg tab. (betahistine 2 HCl), Vetminth oral paste 24 g + 3 g/100 mL (niclozamide, oxybendazole), Vigantol 0.5 mg/mL oral drops (colecalciferol), Vitamin C – monovitan 100 mg tab. (ascorbic acid).

Initial screening of antimicrobial activity

The sterile blotting-paper disks were soaked with 10% (v/v or w/v) solutions of tested drugs in 0.08 M phosphate buffer pH 7 and placed onto Mueller-Hinton 2 Agar (BioMerieux). Plates were

Table 1. Antimicrobial activity of selected non-antibiotic drugs.

Pharmaceutical group – active substance (*Pharmaceutical product*)	Minimal inhibitory concentration (MIC) in mg/mL of active substance in drug			
	Strains tested			
	S. aureus ATCC 6538P	*E. coli* ATCC 8739	*P. aeruginosa* ATCC 15442	*C. albicans* ATCC 10231
Antidepressivum* – paroxetine (*Arketis 20 mg tab.*)	2.5	5	–**	–
Antiepilepticum – valproate Na (*Valpro-ratiopharm Chrono 300 mg tab.*)	–	–	–	8.6
Antiosteoporoticum, Inhibitor – risedronate Na (*Norifaz 35 mg tab.*)	–	1.4	1.4	–
Anxiolyticum – hydroxyzine HCl (*Hydroxyzinum espefa 25 mg tab.*)	3	15	–	15
Inhibitor – atomoxetine (*Strattera 60 mg cap.*)	2.6	2.6	13	2.6
Spasmolyticum – bencyclane fumarate (*Halidor 100 mg tab.*)	12.5	12.5	31	31
Vasodilatans – buflomedile HCl (*Buvasodil 150 mg tab.*)	–	60	–	–
Virustaticum – oseltamivir (*Tamiflu 75 mg tab.*)	–	30	–	–

* the names of pharmaceutical groups according to Podlewski et al (20). ** lack of microbial growth inhibition

inoculated with standardized cells suspension 0.5 unit (McFarland scale) of tested strains. The inhibition of bacterial growth was observed as a halo around the disk containing the tested compound. Size of inhibition zone was correlated with the antimicrobial activity of the drug.

Minimal inhibitory concentration (MIC) determination

Solution of appropriate diluted (once, twice etc.) drug in 0.08 M phosphate buffer pH 7.0 was mixed with 19 mL of a liquefied Mueller-Hinton 2 Agar, cooled to 45°C. The suspension of particular strain of density 0.5 unit (McFarland scale) – 2 µL was applied on the agar surface. The lowest concentration of active substance in the tested drug, which totally inhibited growth of examined strain, was evaluated as MIC value and was presented in Table 1.

RESULTS AND DISCUSSION

It was shown that the drugs listed below inhibited growth of at least one of the examined strains: Arketis 20 mg tab. (paroxetine), Buvasodil 150 mg tab. (buflomedile), Halidor 100 mg tab. (bencyclane), Hydroxyzinum espefa 25 mg tab. (hydroxyzine), Norifaz 35 mg tab. (risedronate), Strattera 60 mg cap. (atomoxetine), Tamiflu 75 mg tab. (oseltamivir), Valpro-ratiopharm Chrono 300 mg tab. with longer dissolution (valproate), Vetminth oral paste 24 g + 3 g/100 mL (niclozamide, oxybendazol).

The MIC values of active substances of these drugs for appropriate strains are presented in Table 1. *E. coli*, *S. aureus* and *C. albicans* strains were susceptible to drugs listed above in proportions as follows: 7/9, 5/9 and 4/9, respectively. Atomoxetine and bencyclane showed broad activity spectrum. They inhibited growth of all examined strains (MIC of atomoxetine: 2.6–13 mg/mL; MIC of bencyklan: 12.5–31 mg/mL). The highest activity against Gram-negative bacteria was found for antiosteoporotic risendronate (MIC = 1.4 mg/mL). Antidepressive paroxetine was active against *S. aureus* and *E. coli* (MIC equal 2.5 mg/mL and 5 mg/mL). Antiepileptic valproate inhibited growth of *C. albicans* strain only (MIC 8.6 mg/mL). In twenty-fold diluted solution of herbal drug Inhalol consisting of aromatherapy oils, antiseptic activity was found against: *S. aureus*, *E. coli* and *C. albicans*. Analyzing anthelminthic paste Vethmith – the mixture of active substances: niclozamide and oxybendazol containing sodium benzoate in concentration

0.2%, it was proved that *S. aureus* was susceptible to 1000-fold dilution of this drug. In this concentration, sodium benzoate does not inhibit bacteria strains (14), thus it is rather influence of niclozamide and oxybendazol in concentration 0.24 and 0.03 mg/mL, respectively.

Kristiansen et al. (1, 5, 15) confirmed that non-antibiotic compounds enhanced the *in vitro* activity of certain antibiotics against specific bacteria. For instance, phenothiazines could act as supporting compounds in the treatment of multidrug-resistant Gram-negative rods (16) or omeprazole and nizatidine enhanced the inhibition effect of metronidazole on *Helicobacter pylori* growth (17). Therefore, our further investigations will focus on this type of activity. Moreover, the antimicrobial activity of such non-antibiotic drugs emphasises necessity of neutralization of their activity during the microbial purity testing of pharmaceutical products (18, 19). This is the reason why each product should be validated towards its possible biocidal activity. In this study, we examined tablets and capsules in which preservatives were not added. In case of drugs which contain preservatives such as: Antisedan inj. 5 mg/mL containing methyl p-hydroxybenzoate in concentration 1 mg/mL, Convulex 50 mg/mL syrup with propyl and methyl p-hydroxybenzoate, Milocardin and Kelicardina containing ethanol, they did not inhibit growth of any microorganisms, In these cases, filtration method or neutralization factors are not necessary during microbial purity testing.

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