Review

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Photoinactivation of ESKAPE pathogens: overview of novel therapeutic strategy

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The emergence of antimicrobial drug resistance requires development of alternative therapeutic options. Multidrug-resistant strains of *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii, Pseudomonas aeruginosa* and *Enterobacter* spp. are still the most commonly identified antimicrobial-resistant pathogens. These microorganisms are part of the so-called 'ESKAPE' pathogens to emphasize that they currently cause the majority of hospital acquired infections and effectively 'escape' the effects of antibacterial drugs. Thus, alternative, safer and more efficient antimicrobial strategies are urgently needed, especially against 'ESKAPE' superbugs. Antimicrobial photodynamic inactivation is a therapeutic option used in the treatment of infectious diseases. It is based on a combination of a photosensitizer, light and oxygen to remove highly metabolically active cells.

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Although routinely used antimicrobials have been considered miraculous drugs for the past 80 years, the emergence of antimicrobial drug resistance requires the development of alternative therapeutic options. The drug resistance of microbes is a serious challenge for modern medicine. A dynamic emergence of microorganisms with high drug resistance, in particular resistance to many antibiotics, is observed worldwide. Therefore, it seems necessary to search for complex solutions based on antimicrobial compounds or methods that interact with many cellular targets. Multidrug-resistant (MDR) strains of *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. are still the most commonly identified antimicrobial-resistant pathogens. These microorganisms are part of the so-called 'ESKAPE' pathogens to point out that they are the cause of most cases of hospital infections and express mechanisms that allow them to effectively 'escape' from the action of antibacterial drugs [1]. CDC define few major antimicrobial drug-development needs in case of ESKAPE pathogens: methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *E. faecium* (VRE), fluoroquinolone-resistant *P. aeruginosa*, extended-spectrum β-lactamases (ESBL)-producing and carbapenem-resistant *Enterobacteriaceae* and carbapenem-resistant *Acinetobacter* [2]. Worth noticing that more people now die of MRSA infection than of HIV/AIDS and tuberculosis combined [3]. The emergence of bacterial resistance to many antibiotics poses a serious threat to public health. We have less and less resource of efficient antimicrobial drugs, and in some cases there are no effective treatment options. For more precise control of the spread and accumulation of drug resistance mechanisms among microbes, a clear definition determining the level of drug resistance within microorganisms has been introduced. The proposed classification distinguishes MDR strains, strains with extended drug resistance (extensively drug-resistant) and strains resistant to all currently available antimicrobial drugs (pandrug resistant). This terminology was introduced by the european centre for disease control and prevention (ECDC) to standardize drug resistance profiles of *S. aureus*, *Enterococcus* spp., *Enterobacteriaceae*, *P. aeruginosa* and *Acinetobacter* spp. It is extremely important to be aware that clinicians and scientists are already facing the emergence of panantibiotic-resistant infections. Extremely limited therapeutic options for

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these pathogens force clinicians to employ previously discarded drugs with significant toxicity and lack of data concerning dosage regimen or duration of therapy [4]. In addition, we are witnessing both the growing number of elderly patients as well as patients undergoing serious treatment procedures like surgery, transplantation and chemotherapy, which would finally lead to an increased number of immunocompromised individuals at risk of these infections [5]. Thus, alternative safer and more efficient antimicrobial strategies are urgently needed, especially against 'ESKAPE' superbugs.

Antimicrobial photodynamic inactivation (aPDI) is a therapeutic option used in the treatment of infectious diseases. It is based on a combination of a photosensitizer (PS), light and oxygen to remove highly metabolically active cells, in other words, fungi, viruses or bacteria. Triplet-excited PS is a basic element of the aPDI activity, leading to the formation of singlet oxygen and/or radicals. The action of these reactive oxygen species (ROS) leads to damage to numerous biological molecules and, consequently, to the death of the desired cells [6]. In comparison with other methods of treatment, aPDI has several advantages. Photoactivation allows the local treatment, which reduces the side effects of photodynamic therapy. In addition, aPDI has several cellular targets and therefore it is not biased by development of resistance to the treatment. However, despite such advantages, and increasing knowledge about the effectiveness of photodynamic therapy, it is clear that clinical application of aPDI is still not widespread. Many researchers have focused on finding new or modifying existing PSs in order to maximize the quantum yield of singlet oxygen in the *in vitro* tests, simultaneously studying molecular aspects occurring in microbial cells and the factors affecting different susceptibility of microbes to the photoinactivation. Notably, it is still unclear which are the most important and essential biological targets in photodynamic reaction for achieving effective eradication of microorganisms and which genetic or phenotypic features of microorganisms determine their response to the photoinactivation. In our opinion, reviewing and presenting the successful applications of aPDI against ESKAPE infections is indispensable for the dissemination of its effective clinical use. Starting from the biological effects of photodynamic inactivation, we will discuss studies that exemplify the most pronounced bactericidal effectiveness against ESKAPE pathogens in *in vitro* (both planktonic and biofilm cultures) as well as *in vivo* and clinical trials. We believe that this analysis will help with understanding that developed improved aPDI protocols are promising alternative for infectious diseases treatment.

The main motivation for writing this review is to present reliable studies that could convince medical chemists, microbiologists and clinicians of unquestionable features and the potential of aPDI for the treatment of infectious diseases. In addition, we will also indicate several open questions and challenges, hoping that they will encourage further research in this area.

aPDI against ESKAPE pathogens

Below, the description and discussion of most recent and pronounced studies concerning aPDI efficacy against ESKAPE pathogens is provided. More detailed description of existing findings is presented in appropriate tables (for planktonic cultures, see Table 1; for biofilm-related studies refer to Table 2 and for *in vivo/ ex vivo* studies follow Table 3).

Enterococcus spp.

Enterococcus is a genus of a Gram-positive, facultative anaerobic, catalase-negative and nonspore-forming bacteria. The main pathogens associated with a wide range of clinical infections are *Enterococcus faecalis* and *Enterococcus faecium.* These bacteria can cause wound infections, bacteremia, urinary tract infection (UTI), endodontic infections, endocarditis, intra-abdominal infections and neonatal sepsis [7,8]. Today, an increasing rate of resistance to many groups of antibiotics has been observed, especially to drugs of last resort. Within the *Enterococcus* genus are VRE. Diseases caused by VRE strains could be effectively treated with very limited antimicrobial therapies [7]. Moreover, numerous study reveal that *E. faecalis* plays an important role in endodontic infections and it can be found in the root canal system [9]. This species in the form of biofilm is considered as one of the most resistant species in oral cavity, due to the resistance to conventional antimicrobial agents: chlorhexidine or sodium hypochlorite [10]. Therefore, when routinely used therapeutic options are insufficient, scientists are aimed at research on alternative or complementary treatments. aPDI is a promising option leading to *Enterococcus* spp. eradication. Numerous studies were performed in this field both in case of *in vitro* and *ex vivo* (planktonic and biofilm) cultures. In 2015, Liu *et al.* investigated the effectiveness of two compounds: 5-aminolevulinic acid (5-ALA) and its derivative 5-ALA methyl ester (MAL) against two vancomycin-resistant *E. faecalis* strains employing LED lamp emitting red

Table 1. Antimicrobial photodynamic inactivation of ESKAPE pathogens in planktonic cultures.

Photosensitizer	Light source	Wavelength (nm)	Irradiance (mW/cm^2)	Fluence (J/cm^2)	Max. reduction (log_{10})	Refs.
Enterococcus faecalis						
Porphyrins:						
– 5-ALA/MAL	LED	633	80	288	5.37/5.02	[11]
Phenothiazines:						
– MB	LED	660	153	90	9.98	[87]
– EtNBS + derivatives	Noncoherent	635	50	10	5–7	[23]
Xanthenes:	Dental QTH	380–500	450	108	7.3	[88]
– RB						
Others:						
– Ce6	LED	660	153	60	10.20	[87]
– CUR	LED/Dental QTH	450/380–500	151/450	25/108	10.32/7.65	[87,88]
– Erythrosine	LED	440–480	1200	96	9.55	[12]
– ICG	NIR diode laser	808	2.38	143	5.1	[89]
– Eosin-Y	Dental QTH	380–500	450	108	4.9	[88]
– HYP	LED	590	80	14.4	6.5	[90]
– Bacteriochlorins	NIR	700–850	100	10	> 6	[41]
Staphylococcus aureus						
aBLT	LED	405/400	21/60	306/54–108	4/> 6	[91,42]
Porphyrin:						
– 5-ALA	LED	410	50	164.5	5	[29]
– PpIX	Q. Light PDT lamp	620–780	102	50	~2–4.51	[92]
– $PPArg_2$	LED	627	23.4	20	4.46–5.53	[93]
– Tetra-Py^+-Me	Artificial white light	380–700	4	14.4	>5	[94]
Phenothiazines:						
– EtNBS + derivatives	Noncoherent	635	50	10	~3–6	[23]
– TBO	Diode laser	633	32	6	5.83	[95]
Phthalocyanines:						
– RLP068/Cl	Lumacare lamp	690	100	10	~8	[96]
– Monosubstituted ZnPC/ZnPC	LED	610/627	40/23.4	48	4/3.49–5.77	[93,97]
Xanthenes:	LED	520	23.4	20	3.37–5.47	[93]
– RB						
Fullerenes:						
– Fulleropyrrolidine	Q. Light PDT lamp	385–780	267	160	3.5–> 6	[28]
Others:						
– DIMPy – BODIPY/BODIPYs	Noncoherent	400–700/350–800	65/70	118/21	5–6/>5	[25,31]
– CUR	LED	455	22	37.5	6–9.8	[98]
– HYP	LED	602	14	8	> 3	[99]
– Imidazoacridinone derivative	Q. Light PDT lamp	385–480	100	100	3.2–5	[100]
– NR, NRBr	Visible light source	385–780	~16.8	30.2	~3.7–~5	[24]
– Porphyrin-fullerene C_{60}	Visible light source	350–800	90	162	> 4.5	[101]

aBLT: Antimicrobial blue light treatment; AlGaInP diode laser: Aluminum gallium indium phosphide diode laser; DL: Diode laser; He–Ne laser: Helium–neon laser; HYP: Hypericin; ICG: Indocyanine green; KI: Potassium iodide; LC16: C60 fullerene derivate; LED: Light-emitting diode; ND: No data; NMB: New methylene blue; NIR: Near-infrared; NR: Neutral red; NRBr: Monobrominated neutral red; PpIX: Protoporphyrin IX; $PPArg_2$: Protoporphyrin IX diarginate; PS: Photosensitizer; QTH: Quartz-tungsten-halogen; RB: Rose Bengal; TBO: Toluidine blue ortho; THL: Tungsten-halogen lamp; UVA: Ultraviolet A; ZnPC: Zinc phthalocyanine.

Table 1. Antimicrobial photodynamic inactivation of ESKAPE pathogens in planktonic cultures (cont.).

Photosensitizer	Light source	Wavelength (nm)	Irradiance (mW/cm^2)	Fluence (J/cm^2)	Max. reduction (log_{10})	Refs.
– Radachlorin®	Diode laser	662	213	12/6	6.28/6.1	[95,102]
– Ru(II)-based PS	Noncoherent	530/525	98/50	58.8/100	6.7–8.3/6	[51,103]
– Ru(II) complexes	Dual-wavelength laser	457/532	40	24	~5–~8	[104]
– Ryboflavin derivates	Noncoherent	380–600	50	1.5	6.5–6.6	[22]
Klebsiella pneumoniae						
Porphyrins:						
– Porphyrin paper/PpIX+KI	Noncoherent/LED	400–700/415	65/50	118/10	4/> 6	[33,105]
– ALA/MAL	Noncoherent	400–780	100	360	3.17–3.68/4.3–4.8	[34]
Phenothiazines:	Laser	630	130	39	3	[32]
– TBO/NMB/Azure A						
Phthalocyanines:	Visible light	ND	20.5	45	4.3	[35]
– ZnTM2, 3PyPz						
Others:						
– DIMPy – BODIPY	Noncoherent	400–700	65	118	4–5	[31]
– 2,3-Distyrylindole	Lumacare lamp	ND	–	–	–	[30]
– Vitamin K	UVA	–	–	30	5.8	[36]
Acinetobacter baumannii						
aBLT	LED	400/415	60/19.5	108/70.2	7.06/> 4	[42,106]
Porphyrins:						
– Tetra-Py^+-Me	White lamps (OSRAM)	380–700	40	64.8	6	[43]
– 4I Conjugate	Semiconductor laser	650	–	6	3.77–3.83	[44]
Phenothiazines:						
– EtNBS derivatives	Noncoherent	635	50	10	5–7	[23]
– MB	–	652	100	6	~ 4	[107]
Fullerenes:	Lumacare lamp	400–700	100	120	4	[78]
– LC16 + KI						
Other:						
– Bacteriochlorins	NIR	700–850	100	10	5–6	[41]
– Ryboflavin derivates	Noncoherent	380–600	50	9	> 6	[22]
– DIMPy–BODIPY	Noncoherent	400–700	65	118	4–6	[31]
Pseudomonas aeruginosa						
aBLT:	LED	410/411	60/15.7	108/50	6.55/7	[42,53]
	LED	415	20/19.5	48/109.9	3.54/7.64	[52,108]
Porphyrins:						
– 5-ALA/ALA + EDTA-2Na	LED	635/410	30/164.5	162/9	6.5/4	[55,109]
– TMPyP	LED/THL	525/300–800	50/500	150/210	6/6	[51,110]
– Photofrin/Cl_2PEt	LED	415/420	50/2.8	10/10	7/3	[33,111]
– TAPP/Tetra-Py^+-Me	THL/OSRAM array	350–700/380–700	25.2/4/4	180/43.2/64.8	3/8.1/8	[43,49,112]
Phenothiazines:						
– MB	He–Ne laser/PDT-1200	632.6/560–780	15.2/100	18.2/50	5.6/5	[113,114]
– NMB/TBO	LED/THL	525/300–800	50/500	100/210	3/7	[51,110]

aBLT: Antimicrobial blue light treatment; AlGaInP diode laser: Aluminum gallium indium phosphide diode laser; DL: Diode laser; He–Ne laser: Helium–neon laser; HYP: Hypericin; ICG: Indocyanine green; KI: Potassium iodide; LC16: C60 fullerene derivate; LED: Light-emitting diode; ND: No data; NMB: New methylene blue; NIR: Near-infrared; NR: Neutral red; NRBr: Monobrominated neutral red; PpIX: Protoporphyrin IX; $PPArg_2$: Protoporphyrin IX diarginate; PS: Photosensitizer; QTH: Quartz-tungsten-halogen; RB: Rose Bengal; TBO: Toluidine blue ortho; THL: Tungsten-halogen lamp; UVA: Ultraviolet A; ZnPC: Zinc phthalocyanine.

Table 1. Antimicrobial photodynamic inactivation of ESKAPE pathogens in planktonic cultures (cont.).

Photosensitizer	Light source	Wavelength (nm)	Irradiance (mW/cm^2)	Fluence (J/cm^2)	Max. reduction (log_{10})	Refs.
Phthalocyanines:						
– ZnPc derivative/LuPc-5	THL/DLs and LEDs	<610/665	40 /60	48/50	4/~6	[97,115]
Xanthenes:						
– RB/RB + KI	LED/white lamp	525/540	50/100	150/10	6/7	[51,56]
Others:						
– TLD1411/Phenalen-1-one	LED/noncoherent	525/380–480	50/20	100/1.2	6/5	[51,116]
– BODIPY	THL/noncoherent	400/400–700	47.5/65	171/118	7/4.5	[50,31]
– Ryboflavin derivates	Noncoherent	380–600	50	1.5	6.8	[22]
***Enterobacter* spp.**						
aBLT	LED	400	60	54–108	> 5	[42]
Phenothiazines:	LED/AlGaInP diode laser/LED	660/660/650	25/1428.6/16.7	50/200/ND	5–6	[59,60,117]
– MB						
Xanthenes:						
– RB	LED	460	ND	ND	> 7	[61]

aBLT: Antimicrobial blue light treatment; AlGaInP diode laser: Aluminum gallium indium phosphide diode laser; DL: Diode laser; He–Ne laser: Helium–neon laser; HYP: Hypericin; ICG: Indocyanine green; KI: Potassium iodide; LC16: C60 fullerene derivate; LED: Light-emitting diode; ND: No data; NMB: New methylene blue; NIR: Near-infrared; NR: Neutral red; NRBr: Monobrominated neutral red; PpIX: Protoporphyrin IX; $PPArg_2$: Protoporphyrin IX diarginate; PS: Photosensitizer; QTH: Quartz-tungsten-halogen; RB: Rose Bengal; TBO: Toluidine blue ortho; THL: Tungsten-halogen lamp; UVA: Ultraviolet A; ZnPC: Zinc phthalocyanine.

light (633 ± 10 nm, 288 J/cm^2). Treatment with 5-ALA resulted in 5.37 log_{10} reduction in survival fraction for VRE clinical isolate and 5.22 log_{10} for the reference *E. faecalis* strain. Slightly lower efficiency was observed in case of MAL treatment reaching 5.02 and 4.91 log_{10} reduction in survival fraction for the clinical and reference strains, respectively [11]. Another study concerning *in vitro* planktonic conditions was reported by Borba *et al.*, who described the effectiveness of erythrosine and LED lamp emitting blue wavelength light (440–480 nm). Complete eradication in tested *E. faecalis* strains was observed when cells were exposed to 5 μM erythrosine followed with 240 s of irradiation (~9.6 log_{10} reduction in cell viability) [12]. *In vitro* efficacy of aPDI was also confirmed using biofilm culture conditions. In 2013, Cieplik *et al.* described the aPDI effectiveness toward *E. faecalis* biofilm using newly synthesized PS – SAPYR. *E. faecalis* biofilm formed in 96-well polystyrene culture plates for 72 h and exposed to SAPYR and light irradiation (360–410 nm) revealed significant reduction in cell viability (≥5 log_{10} CFU reduction) [13]. Recently, Diogo *et al.* described aPDI antibiofilm effectiveness wherein four different PSs were tested (rose Bengal, RB; toluidine blue O, TBO; 5,10,15,20-Tetrakis(1-methyl-4-pyridinio)porphyrin tetra(*p*-toluenesulfonate, TMPyP and modified chlorophyll – Zn(II)e_6Me). The highest efficacy reaching approximately 1 log_{10} reduction in viable cell was reported in case of RB activated with green 557 nm light (3780 J/cm^2) and of TBO/TMPyP/Zn(II)e_6Me activated with red 627 nm light (3150 J/cm^2) [14]. Similar results with reduction in cell viability by <1 log_{10} CFU/ml were obtained for TBO against enterococcal biofilm and reported by López-Jimènez *et al.* Higher antibiofilm activity was demonstrated using diode laser light in combination with methylene blue (MB), reaching reduction of *E. faecalis* biofilm by approximately 1–2 log_{10} CFU/ml [15]. *In vitro* aPDI efficacy was also confirmed using *ex vivo* model. Cieplik *et al.* designed a tooth model that included a human premolar, first and second molar. The glass tube containig *E. faecalis* reference strain and PS was placed in the distal root canal of presented model. TMPyP and MB were tested in the same concentrations (10 μM) and the light doses were 2.4 and 4.54 J/cm^2 in case of TMPyP and MB, respectively. Obtained results indicated that application of blue light and TMPyP is slightly more effective (6.5 log_{10} CFU) than red light and MB (5.8 log_{10} CFU) to effectively eradicate *E. faecalis* in single tooth and whole tooth model [16]. Another study employing human tooth model was conducted by Tennert *et al.* in 2014. In this study human front teeth and premolars were selected. Root canals of all teeth were infected with clinical isolate of *E. faecalis* for 72 h to biofilm formation. The effectiveness of aPDI treatment was checked for both primary and secondary endodontic infections. Application of TBO and exposure to irradiation with red light (635 nm) resulted in cell viability reduction >1 log_{10} CFU/ml in primary infections and

Table 2. Antimicrobial photodynamic inactivation of ESKAPE pathogens in biofilm cultures.

Photosensitizer	Light source	Wavelength (nm)	Irradiance (mW/cm^2)	Fluence (J/cm^2)	Biofilm formation method	Max. reduction (log_{10})	Ref.
Enterococcus faecalis							
Porphyrins:							
– TMPyP	LED	627	35	3150	96-well plates, 48 h, 37°C, without agitation	<1	[14]
Phenothiazines:							
– TBO							
–	LED	628/627	ND/35	106/3150	24-well plates, 24 h, 37°C, shaking (60 rpm)/96-well plates, 48 h, 37°C, without agitation	<1	[14,15]
– MB	DL	670	ND	271	24-well plates, 24 h, 37°C, shaking (60 rpm)	>1	[15]
Xanthenes:							
– RB	LED	557	42	3780	96-well plates, 48 h, 37°C, without agitation	<1	[14]
Others:							
– Zn(II)e_6Me	LED	627	35	3150	96-well plates, 48 h, 37°C, without agitation	<1	[14]
– SAPYR	Noncoherent	360–410	600	ND	96-well plates, 72 h, 37°C	≥5	[13]
Staphylococcus aureus							
aBLT	LED	400/455/405	60/75/1.05	162/45/63	PEGs-lids, static, 72 h, 33°C/24-well plates with compact bone, 14 days, 35°C/12-well plates with titanium discs, 48 h, 35°C	<1/3.2/1.55	[42,118,119]
Phenothiazines:							
– MB	InGaAlP laser/DL	660/635	400/1.41	257/84.6	24-well plates with compact and cancellous bone, 14 days, 37°C/12-well plates with titanium discs, 48 h, 35°C	3.06/2.43	[27,119]
– TBO	InGaAlP laser	660	400	257	24-well plates containing compact and cancellous bone, 14 days, 37°C	>2	[27]
Phthalocyanine:							
– RLP068/Cl	DL	689	120	60	6-well plates containing titanium discs, 72 h, aerobic conditions, 37°C	~1.5	[120]
Others:							
– HYP	LED	602	14	25	24-well plates, 24 h, 37°C	2.3–3.5	[99]
– Ru(II) complex 3	Dual-wavelength laser	457 and 532	40	24	96-well plates, 24 h, 37°C	~1	[104]
– MG	InGaAlP laser	660 nm	400	428.5/257	24-well plates containing compact and cancellous bone, 14 days, 37°C	3–4/4.46	[27,118]
Klebsiella pneumoniae							
Porphyrins:							
– 5-ALA, MAL	White	400–780	100	360	24-well plate, 24 h, 37°C	3.49–4.25	[34]
Phenothiazines:							
– TBO, Azure A, NMB	Laser	630	130	39	96-well plates, 48 h, 37°C	<1	[32]
Acinetobacter baumannii							
aBLT	LED	415/400	100/60	432/216	Microtiter plate, static 24 or 72 h, 37°C/pegs-lids, static, 72 h, 33°C	3.18/<1.5	[42,45]

aBLT: Antimicrobial blue light treatment; AlGaInP diode laser: Aluminum gallium indium phosphide diode laser; DL: Diode laser; InGaAlP laser: Indium–gallium–aluminum phosphide laser; LED: Light-emitting diode; ND: No data; THL: Tungsten-halogen lamp.

Table 2. Antimicrobial photodynamic inactivation of ESKAPE pathogens in biofilm cultures (cont.).

Photosensitizer	Light source	Wavelength (nm)	Irradiance (mW/cm^2)	Fluence (J/cm^2)	Biofilm formation method	Max. reduction (log_{10})	Ref.
Pseudomonas aeruginosa							
aBLT	LED	400/415	60/100	108/432	PEG-lids, static, 72 h, 33°C/microtiter plate, static 24 or 72 h, 37°C	<1/3.02	[42,45]
Porphyrin:							
– TAPP/Tetra-Py$^+$-Me	THL/OSRAM lamps	350–700/380–700	25.2/4	180/21.6	Microtiter plates, 24 h, 37°C static/stirring (130 rpm)	3/2.8	[49,112]
Phenothiazines:							
– MB	Nonthermal laser	670	150	72	Maxillary sinus model, shaking, 24 h, 35°C	3.9	[26]
Others:							
– GD11 – BODIPY	THL	400	48	171	PEG-lids, static, 24 h, 37°C	4	[50]
***Enterobacter* spp.**							
aBLT	LED	400	60	162	PEG-lids, static, 72 h, 33°C	<1	[42]

aBLT: Antimicrobial blue light treatment; AlGaInP diode laser: Aluminum gallium indium phosphide diode laser; DL: Diode laser; InGaAlP laser: Indium–gallium–aluminum phosphide laser; LED: Light-emitting diode; ND: No data; THL: Tungsten-halogen lamp.

3 log_{10} CFU/ml in secondary infections, respectively [17]. Above-mentioned studies clearly indicate that aPDI could effectively inactivate *Enterococcus* spp. and serve as alternative treatment option against enterococcal infections.

Staphylococcus aureus

S. aureus, mainly MRSA is one of the most important MDR human pathogen. The number of MRSA infections, both hospital and community acquired, is growing worldwide and they are frequently difficult to treat [18,19]. More than 90% of *S. aureus* clinical isolates are resistant to penicillin and 60% of them are MRSA [20]. 60 years after the introduction of vancomycin strains of reduced susceptibility or even resistant to glycopeptides are reported [21]. Due to the ability of bacteria to rapid acquisition of antibiotic resistance scientists are facing with great need of new therapies development. One of a very promising, noninvasive therapeutic option to cure *S. aureus* infections is aPDI. In the last 5 years, many conventional and novel PSs derivatives with appropriate wavelength light absorption were tested *in vitro* and *in vivo.* Maisch *et al.* synthesized two new flavin derivatives (FLASH-01a and FLASH-07a). These PSs were very effective against MRSA when irradiated with noncoherent light source (380–600 nm, 50 mW/cm^2) resulting in 5–6 log_{10} reduction of viable cells [22]. Other examples of novel PSs are 5-(Ethylamino)-9-diethylaminobenzo[a]phenothiazinium chloride (EtNBS) derivatives synthesized by Vecchio *et al.* Five out of six were very successful in decreasing *S. aureus* viability (3–6 log_{10} CFU/ml) when employing red light (635 nm) at fluence 10 J/cm^2 and irradiance of 50 mW/cm^2 [23]. Next, studies by Urrutia *et al.* describe the synthesis of monobrominated derivative of neutral red (NRBr). Phototoxicity of NRBr was higher than that of NR and effected in complete eradication of MRSA after 30 min of illumination [24]. Very interesting findings were reported by Agazzi *et al.*, who synthesized two cationic derivatives of 2,6-diiodo-1,3,5,7-tetramethyl-8-(*N*-benzyl-4-pyridyl)-4,4′-difluoroboradiazaindacene (BODIPY) and revealed that exposure of *S. aureus* to visible light for 5 min resulted in significant reduction in cell survival (>5 log_{10} CFU/ml) [25]. Considering biofilm conditions, Biel *et al.* conducted experiments where MRSA biofilms were grown in an anatomically accurate maxillary sinus model to mimic *in vivo* conditions of chronic rhinosinusitis. Indicated biofilms were illuminated with 670 nm nonthermal laser light using Sinuwave® ballon catherer in the presence of MB. A single 0.03% MB aPDI treatment, at irradiance of 50 mW/cm^2, for 8 min, resulted in a 4.1 log_{10} CFU reduction [26]. In another biofilm-related study, the effectiveness of aPDI with 660-nm laser combined with MB, TBO and malachite green (MG) dyes against MRSA biofilms formed in compact and cancellous bones was tested [27]. After incubation for 14 days in the 24-well plates biofilms were illuminated with light fluence of 257 J/cm^2 and irradiance of 400 mW/cm^2. All experimental treatments resulted in significant reduction in CFU/ml log_{10} in comparison to the control group. For the compact bones, the highest therapeutic effect was observed in case of MG dye, resulting in 4.46 log_{10} reduction, while for the cancellous groups in case of MB (3.06 log_{10} reduction) [27]. The antimicrobial effectiveness of aPDI

Table 3. Antimicrobial photodynamic inactivation of ESKAPE pathogens in *in vivo*/*ex vivo* studies.

Photosensitizer	Light source	Wavelength (nm)	Irradiance (mW/cm^2)	Fluence (J/cm^2)	Model characteristics	Interval between microbial inoculation and aPDI	Max. reduction (log_{10})	Ref.
Enterococcus faecalis								
Porphyrins:								
– TMPyP	BlueV prototype	400–450	20	2.4	Human tooth	ND	6.5	[16]
Phenothiazines:								
– MB	PDT 1200L	600–700	37.8	4.54	Human tooth	ND	5.8–7.2	[16]
– TBO/TBO	LED/LED	635/628	ND/2000	ND	Human root canals	72/72 h	3/1–2	[17,121]
Staphylococcus aureus								
Porphyrin:								
– 5-ALA	LED	410	50	164.5	Mouse cutaneous ulcers	2 days	2	[29]
Phenothiazines:								
– MB	Nonthermal laser	670	150	72	Maxilary sinus model	24 h	3.1	[26]
Phthalocyanine:								
– RLP068/Cl	Lumacare lamp	690	100	84	Mouse skin abrasions	30 min	2.9	[96]
Fullerenes:								
– Fulleropyrrolidine	LED	525	50	100	Mouse infected wound	30 min	2	[28]
Others:								
– Ru(II)-based TLD1411	Noncoherent	525	50	100	Mouse infected wound	30 min	<1	[51]
Acinetobacter baumannii								
aBLT	LED	415	100/14.6	360–540/55.8	Mouse burn wound	24 h, 48 h/30 min	3/4.4	[45,106]
Porphyrins:								
– 4I Conjugate	Laser	650	ND	50	Full-thickness excision wound	30 min	3.94	[44]
Phenothiazines:								
– MB	–	652	100	108	Mouse skin abrasion wounds	ND	~ 1	[107]
Fullerenes:								
– LC 16	Lumacare lamp	700–850	100	120	Mouse skin abrasion wounds	30 min	4	[78]
Pseudomonas aeruginosa								
aBLT	LED	415	100	304/144	Rabbit keratitis/mouse keratitis	24 h	3/2.5	[122]
aBLT	LED	415	14.6/100	55.8/48	Mouse burn/skin abrasion	30 min	3.5/5	[52,108]
Porphyrin:								
– ALA/ALA + EDTA-2Na	LED	630/410	ND/164.5	80/9	Human/mouse skin ulcers	ND/2 days	~2/5	[55,123]
Xanthene:								
– RB + KI	Lumacare lamp	540	100	20	Mouse skin abrasion wounds	30 min	6	[56]
Others:								
– TLD1411	LED	525	50	100	Mouse cut wound	30 min	<1	[51]

4I Conjugate: 5,10,15,20-Trakis(4-((s)-2,6-diaminohexanamido)-phenyl) porphyrin; 5-ALA: 5-Aminolevulinic acid; aBLT: Antimicrobial blue light treatment; EDTA-2Na: Ethylenediaminetetraacetic acid disodium salt; KI: Potassium iodide; LC16: C60 fullerene derivate; LED: Light-emitting diode; MB: Methylene blue; ND: No data; RB: Rose Bengal; RLP068/Cl: Tetracationic Zn(II) phthalocyanine chloride; TBO: Toluidine blue ortho; TLD1411: [Ru(2,2′-bipyridine)2(2-(2′,2″:5″,2‴-terthiophene)-imidazo[4,5-f] [1,10] phenanthroline)]2+; TMPyP: 5,10,15,20-Tetrakis(1-methyl-4-pyridinio)porphyrin tetra(*p*-toluenesulfonate).

toward *S. aureus* has also been demonstrated in numerous *in vivo* studies. Our group examined *in vitro* efficacy of a C_{60} fullerene derivative (fulleropyrrolidine)-mediated irradiation with white light. Illumination of MRSA samples was performed using visible light source over wavelength range of 385–780 nm (irradiance 267 mW/cm^2, fluence 160 J/cm^2) that effected with significant photoinactivation of *S. aureus* (3.5 to >6 $\log_{10}$ unit reduction). Afterward, *in vivo* experiments using bioluminescent MRSA strain were performed, to analyze if high *in vitro* effectiveness could be translated into *in vivo* activity. In the aPDI-treated mice, the therapeutic effect was observed: after 24-h postirradiation bioluminescent signal decreased dramatically, reaching background level [28]. Another impressive example of *in vivo* studies was performed by Morimoto *et al*. Mouse model of MRSA-infected ulcer was used to test the effectiveness of 5-ALA-mediated aPDI. On each ulcer, the silicone splint was placed to center the wound and to minimize contracture. The ulcers were next inoculated with MRSA (10^{10} CFU/cm^2) and an occlusive dressing was covered. One day after infection, 5-ALA was administered via intraperitoneal injection. Then, photoinactivation using LED with major wavelength of 410 nm (irradiation of 164.5 mW/cm^2, fluence of 50 J/cm^2) was performed every day. After 7 days, post-aPDI the viable bacteria in ulcer tissue was counted and 2 $\log_{10}$ units of reduction was observed when compared with nontreated control [29]. As indicated above, both planktonic as well as biofilm and *in vivo* studies confirm that aPDI could be considered effective therapeutic option against staphylococcal infections.

Klebsiella spp.

Klebsiella pneumoniae is an opportunistic pathogen causing hospital-acquired and community-acquired infections, mainly UTIs and pneumonia. The global concern related to this microbial species focuses on ESBL and carbapenemase-producing strains. There are several known types of *K. pneumoniae carbapenemase* (KPC) conferring moderate to high (KPC-1) or high resistance to carbapenems (KPC-2, KPC-3). Also, recently identified metallo-lactamases with an example of New Delhi metallo-β-lactamase-1 (NMD-1) have been reported first on Indian subcontinent, and further in Europe, Middle East, Africa, Asia and America, exemplifying fast spreading of antimicrobial resistance. The whole doomed scenario is filled by the production of several virulence factors by this species, including capsule polysaccharide, aerobactin, fimbriae and outer membrane protein A, all playing a critical role in mammalian cell invasion. The success in causing UTI and infecting respiratory tract is attributed to the ability of forming a biofilm, which results in dramatic increase in resistance to currently available antimicrobial agents. The whole picture is even more complex as *K. pneumoniae* prefers to form mixed species biofilm as compared to isolated one. For example, *K. pneumoniae* is able to form stable biofilm with *P. aeruginosa*, where specific spatial distribution of each species was reported. Several groups have shown mixed *K. pneumoniae* biofilms with *Proteus mirabilis*, *Candida albicans*, *Streptococcus*, however, the complexity of the interactions in such interspecies biofilms still awaits to be resolved.

As a first-line antibiotic for the treatment of the most dangerous carbapenem-resistant *K. pneumoniae*, high doses of carbapenems are applied, or the same combined with a range of other antibiotics, such as colistin, tigecycline, gentamicin or fosfomycin (second-line antibiotics). Some new generation cephalosporins have shown potency against carbapenem-resistant *K. pneumoniae in vitro*, when combined with tazobactam. However, alternative control methods, such as aPDI, should be proposed along with the discovery of new antibiotics or mixtures thereof. It has been not many years since aPDI started to be widely explored on ESKAPE species, including *K. pneumoniae.* There have been several types of light-activated compounds investigated with respect to *K. pneumoniae* photoinactivation: BODIPY, phenothiazines, porphyrin derivatives and their precursors (5-ALA, MAL), phthalocyanines. Recently, an interesting type of molecules was studied – indole derivatives that present a new structural scaffold for aPDI application. The activity of these compounds against Gram-negative species was only possible in the presence of polymyxin E [30]. From the newest scaffolds studied in terms of antimicrobial activity, boron dipyrromethene (BODIPY)-based compounds present an attractive option, as they exhibit several characteristics attractive for aPDI, including stability at physiological conditions. With the use of DIMPy-BODIPY (2.5 μM), approximately 4$\log_{10}$ reduction in viable *K. pneumoniae* cells was obtained. One of the drawbacks of DIMPy-BODIPY is their relatively low $^1O_2$ production accounting for 11% [31], which seems to be most desired ROS in terms of aPDI application. More commonly studied in aPDI are phenothiazines, which in case of *K. pneumoniae* do not present spectacular results (~3 $\log_{10}$ reduction) [32]. One has to remember, however, that even these PSs that do not significantly reduce the number of bacterial cells but possess good photochemical properties, can become efficient 'killers' when applied together with nontoxic compounds, like potassium iodide (KI). The Photophrin, a US FDA-approved drug for treating cancers, can become efficient against *K. pneumoniae* in the presence of KI (~6 $\log_{10}$ reduction in viable *K. pneumoniae* cells) [33]. From the representatives of FDA-approved drugs, 5-ALA or MAL constitute

an attractive option of PSs as their application resulted in more than 3 log_{10} after application of 10 mM ALA. Of interest, ESBL-producing and ESBL-nonproducing strains were killed with similar efficacy. In case of MAL applications, the results were even better, and exceeded the value of 4 log_{10} reduction in cell number [34]. The 5-ALA or MAL-based aPDI application seems to be attractive from the clinical point of view as beside the fact of the FDA/EMEA approval, these PSs can be used along with white light, which constitutes universal light source. Considering great difficulties for treatment of carbapenem-resistant *K. pnaumoniae* (KPC) an interesting PS is Zn(II) tetramethyltetrapyridinol(2,3-b:2′,3′-g:2″,3″-l:2‴,3‴-q)porphyrazinium salt (ZnTM2,3PyPz). This phthalocyanine reduced the bacterial cell number by 4.3 logs (3 μM). Given the fact that phthalocyanines are poorly water soluble, ZnTM2,3PyPz showed relatively high photokilling properties in aqueous solution [35]. An interesting aPDI option was recently studied with the use of vitamin K5 as a PS. Combination of vitamin K5 and UVA resulted in 7 logs decrease in *K. pnaumoniae* survival, but also in other Gram-negative species [36]. The only drawback of the study seems to be the application of UVA, which is known to negatively influence the DNA structure.

Only two experimental work concerns biofilm analysis upon aPDI treatment: with the use of 5-ALA/MAL and phenothiazines. Whereas in the first case appreciable efficacy of approximately 4 log_{10} reduction (10 mM MAL) was obtained for both ESBL-producing and ESBL-nonproducing clinical isolate [34], there was only slight antibiofilm activity for each of phenothazines studied (TBO; Azure A, AA; and new methylene blue, NMB) [32].

Recently, a very interesting *in vivo* study was published focusing on treating reptile infections. *K. pneumoniae*, resistant to ten antibiotics, was identified as one of the species at the infection site. After 3-month aPDI treatment (once a week) with MB and red laser, all animals (three) improved and showed reduction of inflammatory signs, although signs of reinfections on the first month was also observable [37].

Acinetobacter baumannii

In 2017, WHO presented the list of pathogens for which new antimicrobial agents are urgently needed, therefore, it included for the highest – critical priority carbapenem-resistant *A. baumannii.* This Gram-negative, nonfermentative, catalase-positive coccobacilli is well distributed in environment and it is known as an etiological agent of hospital-acquired infections [38,39]. Due to increasing and fast developing resistance to routinely applied antibiotics, this pathogen is widely responsible for skin, bloodstream and UTIs, especially in intensive care units [40]. Alternative method of fighting with this opportunistic pathogen can be aPDI. Its antimicrobial effectiveness was repeatedly presented in *in vitro* and *in vivo* studies performed for biofilm and planktonic cultures. Most of the studies refer to photoinactivation of *A. baumannii* planktonic cultures with the administration of exogenous PSs. Studies by Huang *et al.* presented the effectiveness of aPDI employing cationic bacteriochlorins (BC37, BC38, BC39) that are monosubstituted PSs [41]. Phototreatment with mentioned bacteriochlorins in concentration ranging from 1 to 5 μM and light fluence 10 J/cm^2 (λ: 750–800 nm) gave significant reduction in viable cell count, varying from 5 to 6 log_{10} units. Next, aPDI efficacy employing blue light and endogenously produced porphyrin sensitizers was presented by Halstead *et al.* for eradication of 12 clinical isolates of *A. baumannii.* This antimicrobial blue light treatment (aBLT; λ: 400 ± 8.5 nm) with light fluence of 108 J/cm^2 resulted in reduction of bacterial viability ranging from 5.8 to 7.6 log_{10} for all tested isolates [42]. Another significant reduction of *A. baumannii* viability was reported by Almeida *et al.* in response to bacterial exposure to white light (light fluence 64.8 J/cm^2) in the presence of Tetra-Py^+-Me porphyrin [43]. The viability of *A. baumannii* isolated from both hospital wastewater and nonsurgical wounds was reduced by 6 log_{10} upon aPDI treatment [43]. Next, porphyrin derivatives containing 4 lysine residues (4I) and irradiated with red light were confirmed to be effective against planktonic cultures of *A. baumannii* representing different profiles of drug resistance. aPDI employing 4I and red light fluence of 6 J/cm^2 resulted in 3.77 and 3.83 log_{10} unit reduction in viable cell count, for MDR and wild-type *A. baumannii* strains, respectively [44]. It is also worth mentioning that phenothiazine versus (exemplified with nile blue derivatives, EtNBS) activated with red light exerted significant antimicrobial effect toward planktonic *Acinetobacter* spp. cultures. The most bactericidal were three out of seven tested derivatives: EtNBS, EtNBS-Ac and EtNBS-G. In this case, red light irradiation resulted in reduced viable cells by 6, 5 and 7 log_{10}, respectively [23]. The antimicrobial efficacy of aPDI was further presented by Maisch *et al.*, who utilized riboflavin derivatives for phototreatment [22]. Two cationic riboflavin derivatives, namely FLASH- 01a and FLASH-07a, consisting of a different number of positive charges were irradiated with light fluencies of 9 and 4.5 J/cm^2, respectively. aPDI with these derivatives resulted in 6.6 and 6.7 log_{10} reduction in viable count for FLASH-01a and FLaSH-07a, respectively [22]. Furthermore, the efficacy of aPDI against *A. baumannii* was proved for biofilm cultures. In 2016, Halstead *et al.* using aBLT (216 J/cm^2)

reported *A. baumannii* inactivation by approximately 1.5 $\log_{10}$ units when grown in biofilm culture [42]. This efficacy was further enhanced by Wang *et al.* in 2016 who employed aBLT (432 J/cm^2) against mature (24 and 72 h old) biofilm culture of bioluminescent *A. baumannii* strain indicating the reduction of cell viability by 3.59 and 3.18 $\log_{10,}$ in case of 24- and 72-h old biofilms, respectively [45]. Additionally, these findings were confirmed using mouse model of *A. baumannii* burn wound infection. Interestingly, microbial biofilm was allowed to grow in mice wounds for 24 or 48 h followed by blue light irradiation. In case of 24 h old biofilm, the applied light fluence was 360 and 540 J/cm^2 for 48-h old biofilm culture. Biofilms exposed to indicated light fluencies resulted in almost complete elimination of bioluminescence signal indicating the cell viability reduction by 3 $\log_{10}$ in comparison to control group [45]. Another valuable example of *in vivo* studies concerning aPDI against *A. baumannii* was described by Yuan *et al.* in 2017. Employing mouse model of full-thickness excisional wound, infected with multidrug resistant strain, Yuan *et al.* proved the high bactericidal effectiveness of amino-acid porphyrin conjugate (4I) [44]. In this case, aPDI exerted reduction of 2.89 $\log_{10}$ in cell viability when tested immediately after aPDI treatment. This efficacy was further increased to 3.94 $\log_{10}$ of reduction when tested at 4th day of experiment [44]. The described above studies of aPDI inactivation of *A. baumannii* utilizing different light sources, various PSs and culture conditions indicate that aPDI could be potent alternative when searching for novel therapeutic option against drug-resistant *Acinetobacter* spp.

Pseudomonas aeruginosa

P. aeruginosa is the second most commonly reported Gram-negative pathogen associated with UTI in intensive care units [46]. Less frequent, but much more severe are bloodstream infections with carbapenem resistant *P. aeruginosa* attributed to mortality up to 18.4% [47]. Moreover, *P. aeruginosa* can manifest resistance to quinolones, aminoglycosides or polymyxins, as well as multiple drugs (MDR strains) with no drugs available in clinical development [48]. The alternative could be aPDI. The effectiveness of Tetra-Py$^+$-Me porphyrin activated with visible light was assessed by Beirao *et al.,* who reached 8.1 $\log_{10}$ reduction in viable cell count in case of planktonic *P. aeruginosa* culture employing 10 μM concentration of Tetra-Py$^+$-Me and light fluence of 43.2 J/cm^2 [49]. Similar results, 7 $\log_{10}$ reduction, were demonstrated by Orlandi *et al.* with BODIPY PS at low concentration of 2.5 μM and light fluence of 171 J/cm^2 (λ: 400 nm) [50]. Our team also investigated the antimicrobial effectiveness of aPDI with four different PSs activated with visible light (λ: 525 nm) against planktonic *P. aeruginosa* cultures. We reached 6 $\log_{10}$ reduction in viable cell count for RB, TMPyP and Ru(II)-based derivative (TLD1411) [51]. Alternative to exogenously administered aPDI is aBLT employing blue light activation of endogenously produced PSs. The bactericidal effectiveness of aBLT (λ: 400 nm, light fluence 108 J/cm^2) was assessed by Halstead *et al.* against five both reference and clinical *P. aeruginosa* isolates. Obtained results indicated the reduction in cell viability varying from 5.59 to 6.55 $\log_{10}$ units [42]. aBLT (λ: 415 nm) was also investigated by Amin *et al.*, who received 3.54 $\log_{10}$ reduction per cycle in ten cycles of bacterial inactivation and regrowth. Moreover, within mentioned study no evidence of development of *P. aeruginosa* tolerance to aBLT was reported [52]. Our group also investigated aBLT efficacy. We reported 7 $\log_{10}$ unit reduction in case of four studied *P. aeruginosa* isolates by employing blue light (λ: 411 nm) fluence of 50 J/cm^2 [53,54]. aBLT effectiveness against *P. aeruginosa* was also confirmed for biofilm cultures. Halstead *et al.* with the use of aBLT (light fluence 108 J/cm^2) reported approximately 1 $\log_{10}$ reduction of biofilm seeding in case of studied clinical isolates. The influence of aBLT on *P. aeruginosa* biofilm formation was also demonstrated by studies of our group indicating that sublethal doses of aBLT can successfully delay biofilm formation [53]. Worthy of note, the reduction in microbial viability by 2.8 $\log_{10}$ units was also reported by Beirao *et al.*, who used Tetra-Py$^+$-Me porphyrin activated with visible light for *P. aeruginosa* biofilm treatment [49]. Finally, significant antibiofilm activity was presented by Orlandi *et al.,* who employed BODIPY PS for aPDI treatment reaching 4 $\log_{10}$ reduction in viable cells growing in biofilm. Next, to investigate if *in vitro* studies could be easily translated to clinical applications, some *in vivo* models were described. Our team utilized and optimized mouse cut wound model imitating chronic wound infection for aPDI treatment [51]. When using TLD1411, 1 $\log_{10}$ reduction in bioluminescence level associated with delayed infection development was observed [51]. Our most recent studies describe the application of *Caenorhabditis elegans* infection with *P. aeruginosa* to investigate antimicrobial efficacy of aBLT treatment. Performed experiments confirm that sublethal doses of aBLT resulted in increased *C. elegans* survival rate in response to decreased virulence and/or delayed biofilm production upon aBLT exposure [53]. *In vivo* aBLT efficacy was also investigated by Amin *et al.*, who reported 5 $\log_{10}$ reduction of bacterial bioluminescence in case of mouse skin abrasion wound infection [52]. Similar antimicrobial effectiveness was observed by Katayama *et al.*, who reported 5 $\log_{10}$ reduction of bacterial count using 5-ALA-induced production of endogenous porphyrins

activated with 410 nm wavelength light [55]. Another *in vivo* study for aPDI efficacy was performed by Wen *et al.*, who applied RB in the presence of KI activated with 540 nm wavelength light [56]. This application resulted in 6 $\log_{10}$ reduction in *P. aeruginosa* bioluminescence in mouse skin abrasion wounds. Mentioned above studies by Amin *et al.*, Katayama *et al.* and Wan *et al.* suggest that aPDI could actually be considered a promising option for *P. aeruginosa* eradication.

Enterobacter spp.

Enterobacter species are the etiological agents for an increasing number of healthcare-associated infections with emerging and developing resistance to multiple antimicrobials [57]. These infections are characterized with significant morbidity and mortality [58]. As with other members of the *Enterobacteriaceae*, drug resistance occurs via ESBL and carbapenemases as well as chromosomal cephalosporinases [57]. Only few antimicrobials are still active against this microorganism; thus, the emergence of aPDI as an alternative seems attractive. There are only few studies concerning photodynamic efficacy toward *Enterobacter* spp. resulting in significant microbial inactivation. Khan *et al.* reported 5 $\log_{10}$ unit reduction in viable cells when *E. cloacae* was administered with phenothiazine sensitizer, MB, and exposed to light irradiation [59]. Employing the same PS, Carvalho *et al.* reported 6 $\log_{10}$ units reduction in cell viability *in vitro* when *Enterobacter* spp. isolated from infected wounds was treated with aPDI [60]. Finally, using xanthen PS, RB, Rossoni *et al.* reached the *E. cloacae* viability reduction of approximately 7 $\log_{10}$ [61]. In addition, when employing aBLT without administration of exogenous PSs, Halstead *et al.* reported that planktonic-phase bacteria were susceptible to aBLT demonstrating $\geq$5 $\log_{10}$ decrease in viability [62]. The same research group provided the only existing results concerning photodynamic treatment of *Enterobacter* spp. when cultured in mature biofilms. Obtained results indicated that bacterial biofilms were highly susceptible to blue light, with significant reduction in cell viability for all isolates and all aBLT exposures (λ: 400 $\pm$ 8.5 nm) [62]. Unfortunately, no *in vivo*/*ex vivo* studies concerning aPDI *Enterobacter* spp. were published so far, nevertheless, described studies warrant further investigations of aPDI as a novel antimicrobial strategy and indicate that it could be successfully used for *Enterobacter* spp. decontamination applications.

Strategies to potentiate aPDI

Potentiation of aPDI by nanotechnology

Nanotechnology provides an attractive approach leading to improved bactericidal efficacy of aPDI [63–66]. This improvement may result from both enhanced targeted PS delivery to microorganisms via PS-loaded nanoparticles [67] or increased ROS and singlet oxygen quantum yield of the PS via its mixing or covalently binding with nanoparticles [68,69]. In addition, some nanoparticles, in other words, TiO_2 or fullerenes, could express photosensitizing activity resulting in effective inactivation of microorganisms [70]. The most widely investigated material used for production of PS nanoparticles is chitosan that is a biodegradable and naturally occurring polymer. Moreover, increased aPDI efficacy benefits from its intrinsic cationic charge leading to enhanced targeted PS delivery to microbial over eukaryotic cells. The successful employment of chitosan-loaded PS was numerously reported in case of various microbial as well as fungal cells [71–73]. Another, and most recent nanotechnology-based approach leading to enhanced aPDI results from conjugation of PSs to gold nanoparticles. In this case, the potentiation of aPDI was confirmed with the employment of mature MRSA biofilms treated with PS-conjugated gold nanoparticles. This report is of high interest as significant achievement (>4 $\log_{10}$ extra killing) was observed when compared with free PS-based aPDI [74].

Potentiation of aPDI by inorganic salts

Another attractive strategy leading to potentiated aPDI was developed by the group of Michael Hamblin who reported that the administration of inorganic salts to the aPDI-treated samples, results in more than 6 $\log_{10}$ extra killing. The greatest effect was observed in the case of KI [75], but significant enhancement of aPDI efficacy was also reported when potassium bromide [75], sodium azide [76], sodium thiocyanate [77] and sodium nitrite were employed. Huang *et al.* indicated that the mechanism underlying the observed potentiation may result from the action of singlet oxygen leading to the generation of iodine radicals and molecular iodine from iodide [33]. In case of sodium azide, the mechanism may result from the generation of azide radical upon the oxidation of azide anion [76]. Numerous *in vivo* published data supported this potentiation strategy revealing that the aPDI efficacy could, indeed, be significantly improved [75,78,79].

Potentiation of aPDI by antimicrobials

The latest discovery associated with the strategy to enhance the bactericidal effect of aPDI is to demonstrate that aPDI sensitizes microbes to antimicrobials. Numerous studies, both *in vitro* and *in vivo*, using a whole range of different categories of antibiotics and PSs, confirmed that this combined therapy may exert spectacular effects. Undoubtedly, the interaction of aPDI with antimicrobials could be assigned as synergy that leads to the eradication of microorganisms using very low (sublethal) concentrations of both PSs and antimicrobials against that the microbes expressed high resistance. Moreover, despite the enhancement of the bactericidal effect, another important effect that can be expected due to the use of aPDI/antimicrobials combined approach may be shortening the treatment time and reducing the rate of microbial drug resistance development. All these issues as well as all the published studies regarding aPDI/antimicrobials combined treatment, and hypotheses regarding the potential mechanisms underlying this synergistic impact have been deeply analysed and thoroughly reviewed in the latest review by Wozniak *et al.* [80].

Conclusion

This review provides unquestionable evidence for high bactericidal efficacy of light based approaches to be succsessfully employed when fighting against the most threatening microbes – ESKAPE pathogens. It has been evidenced both with *in vitro* (planktonic and biofilm cultures) and *in vivo* studies. Moreover, within the current paper the most potent strategies – nanotechnology, iodide salts and the combination with routinely used antimicrobials – were evidenced to potentiate the bactericidal efficacy of the light based treatments.

Future perspective

The problem of antibiotic resistance has been perceived by global bodies, including European Commission, ECDC, WHO and antimicrobial resistance (AMR) has become a priority in recent years, illustrated in several important reports [81–83]. However, there still there is no change in the approaches dealing with AMR. The current approaches include: appropriate use of antibiotics; strengthening the regulatory framework on veterinary medicine; recommendation to prudent use of drugs; strengthening infection prevention; strengthening the surveillance system and bringing new antibiotics to patients. All the listed points although important and necessary, will not bring long-lasting change in the observed situation. In the case of new antibiotics, unquestionable is the fact that we need new ones, however for those familiar with the rapidness of bacterial evolution, that can be actually observed within days, selection of isolates resistant to new antibiotics is just a matter of time. This is not the case for photoantimicrobials, which act on many targets in bacterial cell. Several bodies of evidence indicated already that the development of resistance in this case is highly unlikely [84–86]. That is why we should focus in the near future on the development and analysis of light-activated multitargeted drugs and bringing the existing ones, for example, MB into clinical practice. Photoantimicrobials and aPDI technology could be easily adapted into main stream clinical practice to reduce AMR level. Although there is a range of PS available, still their action is underappreciated and what is more important research on aPDI is underfunded. Current applications of aPDI concerns local administration of a PS, rather than systemic one, followed by local delivery of light. This secures safety of the technology, however, also limits the possibilities of aPDI applications. Nevertheless, if aPDI could be applied in, for example, wounds decolonization of patients, invasion to the bloodstream and systemic infections could be prevented without application of classical antibiotics. We believe that the future work will be done improving selectivity of PS over host tissue, while sparing the nonselective nature toward microbial species. Ideal PS would be active against both Gram-positive and Gram-negative species, which are known to differently respond to treatment. Another limitation of broader use of photoantimicrobials is lack of mechanistic studies on how PS acts on microbial cells and host cells. Such knowledge is necessary to better design new PS with improved properties, and we believe within next years there will be increased number of studies addressing the problem of molecular basis of aPDI, accordingly as it was done for PDI in cancer cells. Another important issue is light as an indispensable constituent of aPDI action. The way and amount of light is being delivered to the treated place affects aPDI outcome. In recent times, there are several types of light sources available, including lasers and light-emitting diodes for particular applications. Moreover, it would be of great interest to develop a system that allows for the control of light dosimetry at the treated site. The newest aPDI future should be devoted to the development of such an imaging system that would allow therapy to be monitored. From the many *in vivo* experiments on animal models of infections treatment by aPDI, it is known that often a reinfection occurs. Thus, the system for monitoring both, dosimetry and therapy should be developed. Such technological solution would definitely contribute to broadening the application of aPDI in clinical practice.

Executive summary

Increasing resistance to clinically applied antimicrobials is noted, specifically among ESKAPE (*Enterococcus* spp.; *Staphylococcus aureus*; *Klebsiella* spp.; *Acinetobacter baumannii*; *Pseudomonas aeruginosa* and *Enterobacter* spp.) pathogens. Antimicrobial photodynamic inactivation (aPDI) constitutes a very good option for clinical application in the treatment of local infections caused by multidrug-resistant pathogens.
In the presented review, we evidenced the newest proofs for:

aPDI against ESKAPE pathogens

- *Enterococcus* spp. (effective photoinactivation of *Enterococcus* spp., including vancomycin-resistant *E. faecium* strains).
- *Staphylococcus aureus* (effective photoinactivation of *S. aureus*, including methicillin-resistant *S. aureus* strains).
- *Klebsiella* spp. (effective photoinactivation of *K. pneumoniae*, including extended-spectrum β-lactamases-producing strains).
- *Acinetobacter baumannii* (effective photoinactivation of *A. baumannii*, including carbapenem-resistant strains).
- *Pseudomonas aeruginosa* (effective photoinactivation of *P. aeruginosa*, including multidrug-resistant strains).
- *Enterobacter* spp. (effective photoinactivation of *Enterobacter* spp.).

Strategies to potentiate aPDI

- Potentiation of aPDI by nanotechnology.
- Potentiation of aPDI by inorganic salts.
- Potentiation of aPDI by antimicrobials.

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