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Evolution of antimicrobial resistance during the last decade in the European Union

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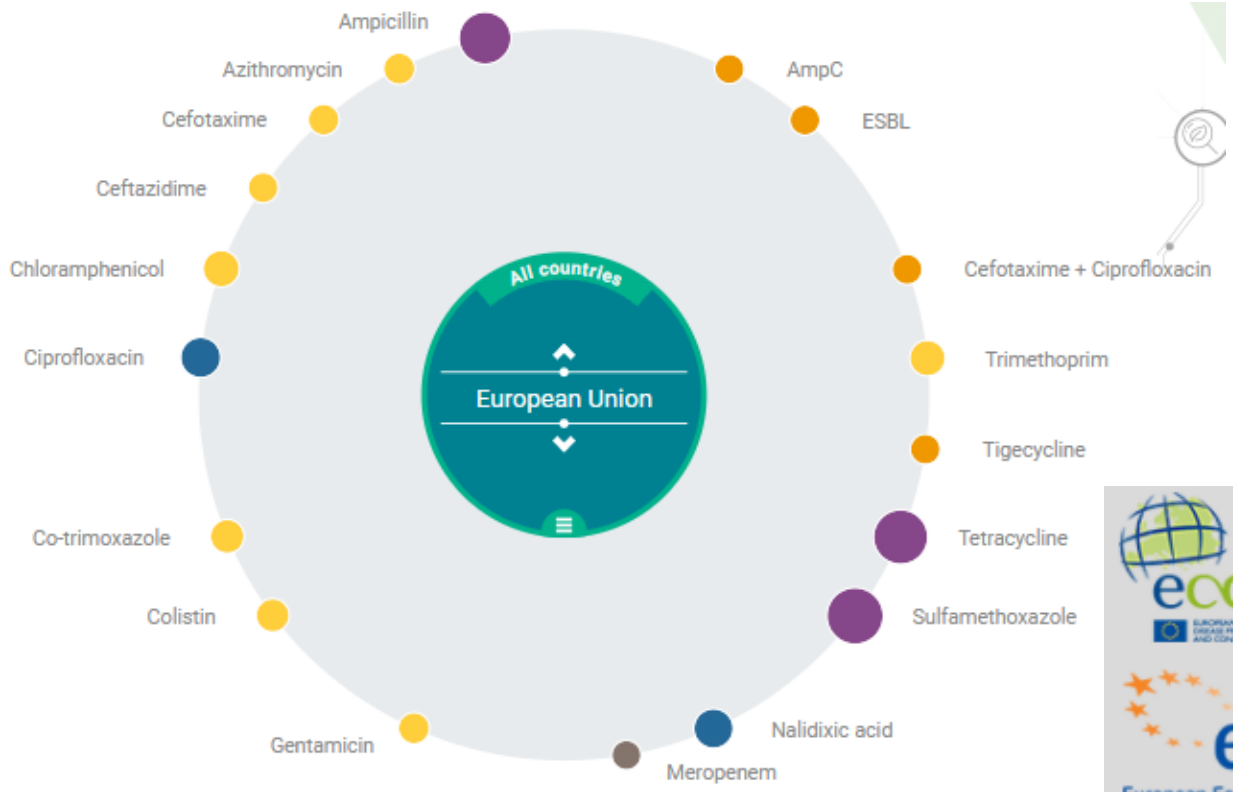
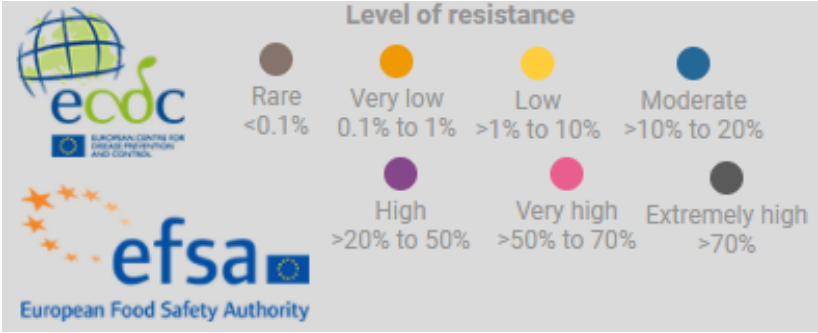
Evolution of antimicrobial resistance during the last decade in the European Union





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Abstract: Nowadays, antimicrobial resistance (AMR) is a global health and development threat with three main causes such as the misuse and overuse of antimicrobial consumption, poor patient adherence to therapies, and the limited numbers of new drugs under development. This work main objective is to illustrate the current antimicrobial resistance situation in the European Union during the last decade and the main challenges to fight it. WHO published a Global Priority Pathogens List comprising twelve antibiotic-resistant priority bacteria that pose the greatest threat to human health with increasing trends observed in *E. coli* and *K. pneumoniae*. *Acinetobacter* spp. possesses one of the highest resistant percentages of isolates while significantly decreasing trends have been observed with *P. aeruginosa*. Other recent concern bacteria are methicillin-resistant *S. aureus* (MRSA) vancomycin-resistant *Enterococci* (VRE) which are associated with an increased risk of infection and mortality. Strategies to fight AMR are the identification of new potential antimicrobial targets and/or of new chemical entities that hit bacterial non-essential targets. Combination therapies of existing antibiotics and smart antibiotic adjuvants are in great demand, however it is challenging as the research and development process is time-consuming requiring investment from the pharmaceutical industry. AMR will continue to be one of the main threats for global health, which will require significant efforts at different social levels. Therefore, the identification of new strategies to limit or to overcome the occurrence of resistance strains will be a long journey, where antibiotic adjuvants counteracting antibiotic resistances will cover a significant area of the AMR fields.

Keywords: antibacterials; antibiotic adjuvants; antimicrobial resistance

Introduction

- current antibacterials in the market
- global priority pathogens list
- current situation in Portugal and the European Union
- strategies and approaches to fight AMR

Introduction – Antimicrobial resistance (AMR)

Main causes:

misuse and overuse of antimicrobial consumption

poor patient adherence to antimicrobial therapies

limited numbers of new drugs under development to replace those ineffective

Introduction – Antibacterials

WHO Collaborating Centre on Global Governance of Antimicrobial Resistance

Table 1- Anatomical Therapeutic Chemical (ATC) classification of antibacterials (WHOCC, 2021)

J01A Tetracyclines	J01B Amphenicols	J01C Beta-lactam antibacterials, penicillins	J01D Other beta-lactam antibacterials	J01E Sulfonamides and trimethoprim
•J01AA Tetracyclines	•J01BA Amphenicols	•J01CA Penicillins with extended spectrum •J01CE β-lactamase-sensitive penicillins •J01CF β-lactamase-resistant penicillins •J01CG β-lactamase inhibitors •J01CR Combinations of penicillins	•J01DB 1st-gen cephalosporins •J01DC 2nd-gen cephalosporins •J01DD 3rd-gen cephalosporins •J01DE 4th-gen cephalosporins •J01DF Monobactams •J01DH Carbapenems •J01DI Other cephalosporins and penems	•J01EA Trimethoprim and deriv. •J01EB Short-acting sulfonamides •J01EC Intermediate-acting sulfonamides •J01ED Long-acting sulfonamides •J01EE Combinations •QJ01EQ Sulfonamides •QJ01EW Combinations

J01F Macrolides, linco. and streptogramins	J01G Aminoglycoside antibacterials	J01M Quinolone antibacterials	J01X Other antibacterials
•J01FA Macrolides •J01FF Lincosamides •J01FG Streptogramins	•J01GA Streptomycins •J01GB Other aminoglycosides	•J01MA Fluoroquinolones •J01MB Other quinolones •QJ01MQ Quinoxalines •J01RA Combinations of antibacterials •QJ01RV Combinations of antibacterials and other subst.	•J01XA Glycopeptide antibacterials •J01XB Polymyxins •J01XC Steroid antibacterials •J01XD Imidazole derivatives •J01XE Nitrofuran derivatives •QJ01XQ Pleuromutilins •J01XX Other antibacterials

Introduction – Global Priority Pathogens List (PPL)

Table 2- WHO Priority Pathogens List (PPL) (WHO, 2017)

Critical	High	Medium
• *Acinetobacter baumannii*, **carbapenem-resistant**	• *Enterococcus faecium*, **vancomycin-resistant**	• *Streptococcus pneumoniae*, **penicillin-non-susceptible**
• *Pseudomonas aeruginosa*, **carbapenem-resistant**	• *Staphylococcus aureus*, **methicillin-resistant and vancomycin intermediate**	• *Haemophilus influenzae*, **ampicillin-resistant**
• *Enterobacteriaceae*, **carbapenem- and 3rd gen. cephalosporin-resistant**	• *Helicobacter pylori*, **clarithromycin-resistant**	• *Shigella* spp. , **fluoroquinolone-resistant**
	• *Campylobacter*, **fluoroquinolone-resistant**	
	• *Salmonella* spp., **fluoroquinolone-resistant**	
	• *Neisseria gonorrhoeae*, **fluoroquinolone- and 3rd-gen. cephalosporin-resistant**	

Introduction – Resistant strategies of bacteria

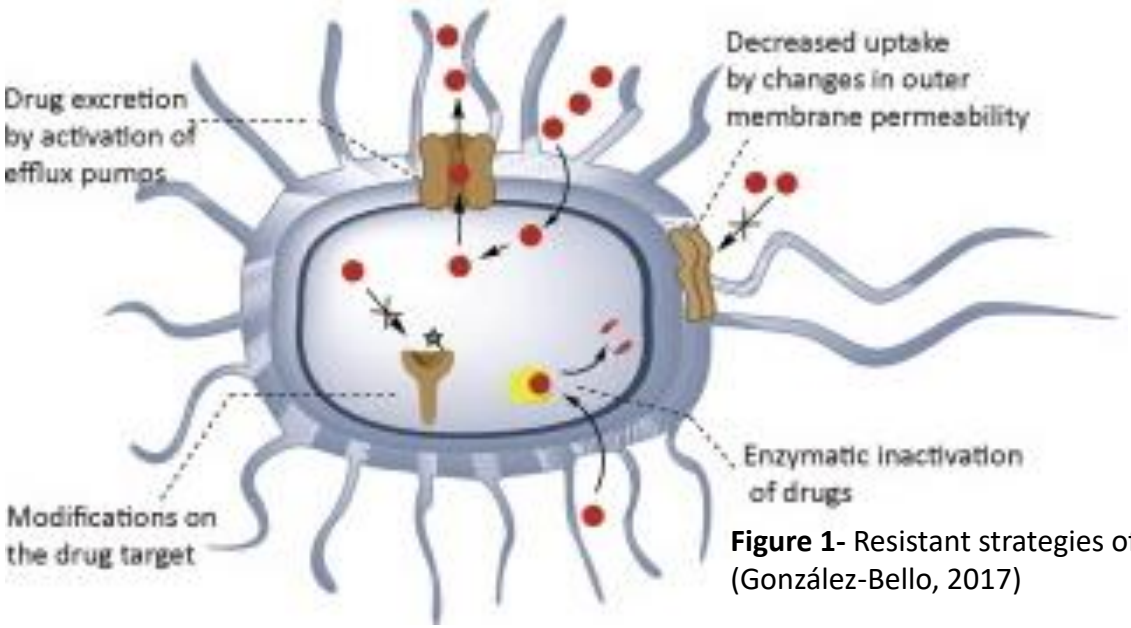


Figure 1- Resistant strategies of bacteria (González-Bello, 2017)

- **poor influx and excessive drug efflux**
- **enzymatic inactivation of drugs**
- **modifications on drug target** by R plasmids
- **biofilms**

Results and discussion
- EU/EEA current situation

- 33,000 deaths /year;
- €1.5 billion annual cost;

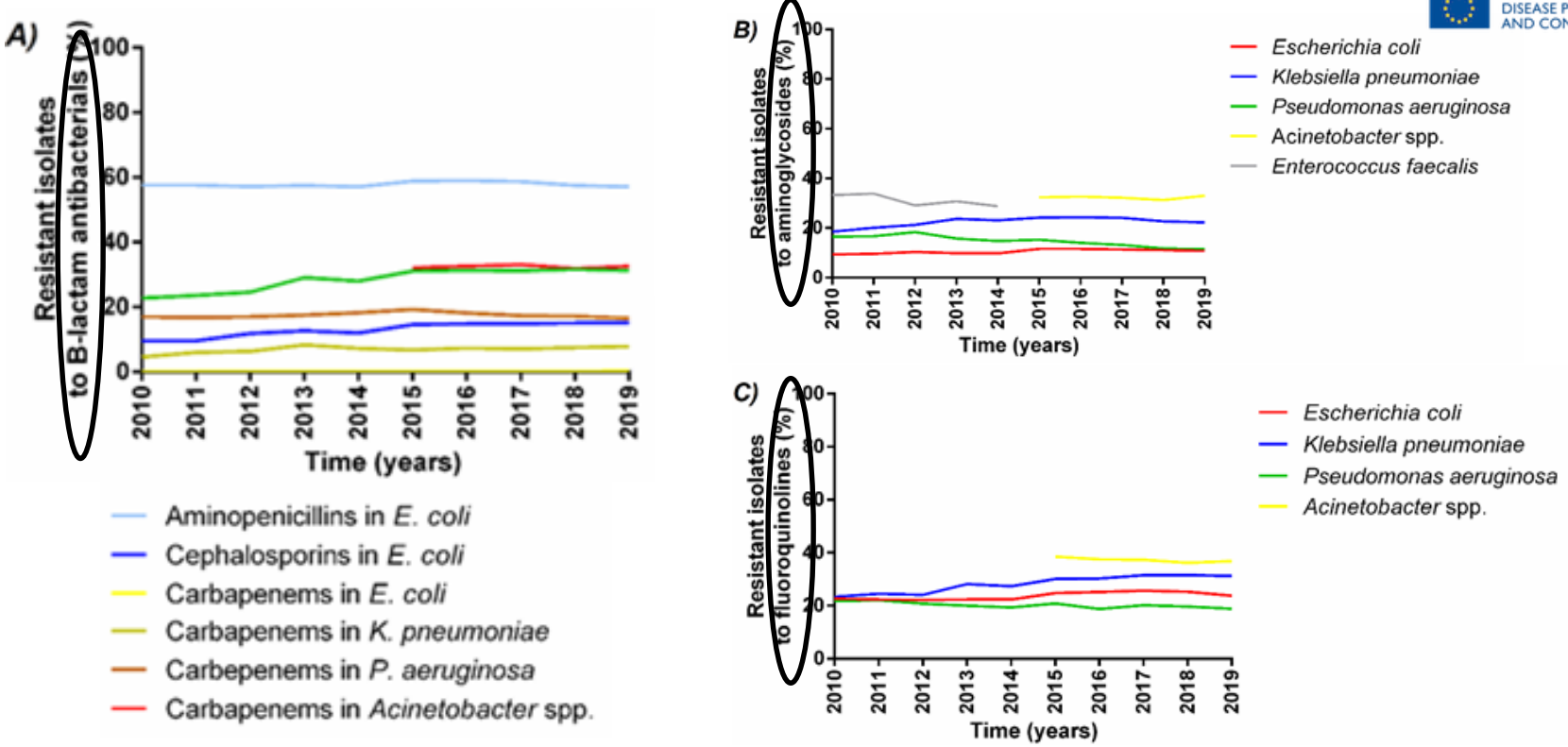


Figure 2- Population-weighted mean AMR percentages (%) for (A) β-**lactam antibacterials**, such as aminopenicillins (amoxicillin/ampicillin), third-generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime), and carbapenems (imipenem/meropenem), (B) **aminoglycosides** (gentamicin/netilmicin/tobramycin) and (C) **fluoroquinolones** (ciprofloxacin/levofloxacin/ofloxacin) in EU/EEA (ECDC, 2020b)

Results and discussion
- EU/EEA current situation

Table 3- Population-weighted mean AMR percentages (%) in Portugal (ECDC, 2021) and in the EU/EEA zone (ECDC, 2020b) for fluoroquinolones (ciprofloxacin/levofloxacin/ofloxacin), aminoglycosides (gentamicin/netilmicin/tobramycin), third-generation cephalosporins (cefotaxime/ceftriaxone/ceftazidime), carbapenems (imipenem/meropenem), aminopenicillins (amoxicillin/ampicillin), vancomycin, methicillin and piperacillin + tazobactam

Resistant isolates in 2019 (%)	Gram positive bacteria						Gram negative bacteria							
	E. faecalis		*E. faecium*		*S. aureus*		*K. pneumoniae*		*A. baumannii*		*P. aeruginosa*		*E. coli*	
	PT	EU	PT	EU	PT	EU	PT	EU	PT	EU	PT	EU	PT	EU
fluoroquinolones							45.8 ↑ **+14.4**	31.2 ↑ **+7.8**	26.1 * ↓ **-51.2**	36.9 ** ↓ -1.6	21.6 ↑ +1.3	18.9 ↑ -2.8	26.5 ↓ -0.8	23.8 ↑ +1.1
aminoglycosides	22.2 ↓ **-16.8**	26.6 ° ↓ **-6.7**	21.8 ↓ **-31.4**				32.2 ↑ +5.6	22.3 ↑ +3.8	24.7 * ↓ **-40.4**	33.0 ** ↑ +0.6	9.9 ↓ -4.0	11.5 ↓ **-5.0**	12.1 = 0.0	10.8 ↑ +1.4
3rd generation cephalosporins							47.6 ↑ **+19.4**	31.3 ↑ **+8.5**			17.6 ↑ **+5.4**	14.3 ↑ +2.4	16.1 ↑ **+5.7**	15.1 ↑ **+5.6**
carbapenems							10.9 ↑ **+9.5**	7.9 ↑ +3.3	31.1 * ↓ **-48.1**	32.6 ** ↑ +0.5	17.8 ↑ +1.7	16.5 ↓ -0.5	0.1 ↓ -0.2	0.3 ↑ +0.3
aminopenicillins	0.5 ↓ **-16.8**		85.0 ↓ **-5.9**										58.5 ↑ +2.7	57.1 ↓ -0.6
vancomycin	0.3 ↓ -1.5		9.0 ↓ **-14.4**	18.3 ↑ **+12.7**										
methicillin					34.8 ↓ **-18.6**	15.5 ↓ **-6.7**								
piperacillin + tazobactam											20.3 ↑ +4.2	16.9 ↑ +1.2		

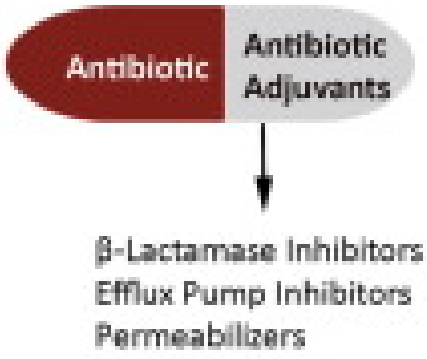
Note: All increases (↑) and decreases (↓) of percentages of resistant isolates were in relation to 2010 AMR data, with the exception of * which was from 2012 and ** which was from 2015.

Results and discussion
- Strategic therapies to combat resistance

antibiotic combinations

synergy between antibiotics and non-antibiotics (**antibiotic adjuvant** therapies)

molecules that alter the physiology of antibiotic-insensitive cells (such as in biofilms)



Results and discussion
- Strategic therapies to combat resistance

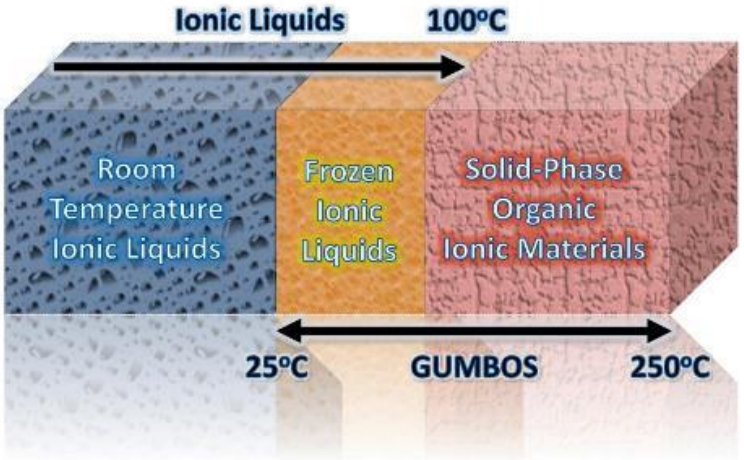
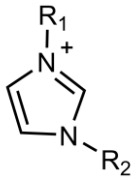


Figure 3- Difference in melting temperatures between ILs and GUMBOS (Warner, 2014).

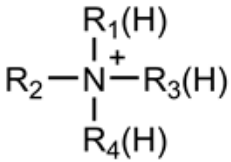
- **Ionic liquids** (**ILs) and organic salts (GUMBOS)** as chemical approaches that could remedy challenges posed by conventional drug combination therapies (Cole, 2013).
    - easily adapted for “designer drugs” as properties can be tuned by choice of counterion (Pedro, 2020).
    - wide range of attributes (MacFarlane, 2002; Rogers & Seddon, 2003).

Results and discussion
- API-ILs as antimicrobial agents



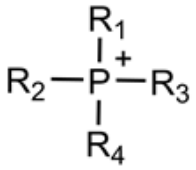
Imidazolium-based ILs:

Anions: [Cl], [Br], [BF_4], [PF_6], [NaI], [NTf_2], [NO_3], [TfO], [OAc], [β-lact], FQs



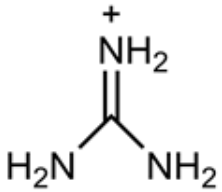
Ammonium, benzalkonium and benzethonium-based ILs:

Anions: [Lac], [Sac], [Ace], [Man], [L-Pro], [NaI], [Theo], [NAA], [Pyr], [β-lact], [AA]



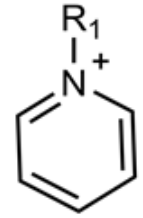
Phosphonium-based ILs:

Anions: [Cl], [Br], [Oleate], [Hexanoate], [Geranate], [NaI], [β-lact], [$N(CN)_2$], [NTf_2], [AA]



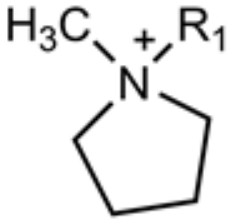
Guanidinium-based ILs:

Anions: [I], [C_2OSO_3]



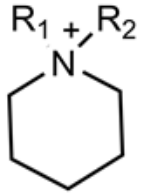
Pyridinium-based ILs:

Anions: [Cl], [Br], [Sac], [Ace], [NO_3], [BF_4], [NTf_2], [β-lact], [FQ]



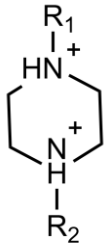
Pyrrolidinium-based ILs:

Anions: [Cl], [Br], [NTf_2], [TfO], [NaI]



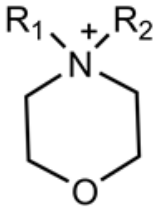
Piperidinium-based ILs:

Anions: [Cl], [Br], [I], [Man], [NaI], [TFSA], [Pyr]



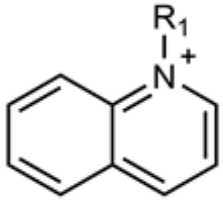
Piperazinium-based ILs:

Anions: [BF_4]$_2$, [BF_4], [Lac]



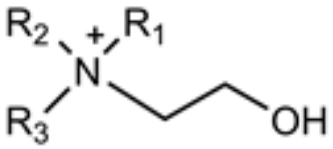
Morpholinium-based ILs:

Anions: [Cl], [NaI], [Pyr], [2,4-D], [4-CPA], [Clopyralid], [Dicamba], [MCPA], [MCPP]



Quinolinium-based ILs:

Anions: [Cl], [I], [B], [TMS], [N_2S]



Cholinium-based ILs:

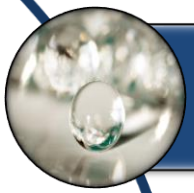
Anions: [AA], [oleate], [hexanoate], [geranate], [malonate], [Cl], vitamins ([Asc], [Biot], [Nicot]), [β-lact], [Nor]

Results and discussion
- API-ILs as antimicrobial agents

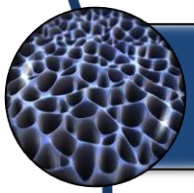
API-based ILs from commercially available APIs:

- **antiseptics** domiphen (Cybulski, 2011) and chlorhexidine (Cole, 2015) as cations;
- **anti-inflammatory** ibuprofenate, **laxative** docusate and **antibiotic** sulfacetamide as anions with **aminoacid** glycine and **histamine-2 blocker** ranitidine as cation (Frizzo, 2016);
- **anticancer** matrine (Z. Wang, 2019) as cation;
- **antibacterial** β-lactams ampicillin (Cole, 2011)(Ferraz, 2014), penicillin and amoxicillin (Ferraz, 2020) as anions and also **antibacterial** fluoroquinolones ciprofloxacin (Osonwa, 2017) as cation and ciprofloxacin and norfloxacin (Santos, 2020) as anions;

Results and discussion
- IL-assisted biomaterials with antimicrobial properties:

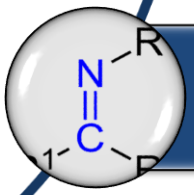


Hydrogels of polymerized ILs, are being researched for their antimicrobial profile (Raucci et al., 2018), (Y. Zhang et al., 2020), (Kayalvizhy et al., 2020);



Combination of ILs with **polymer membranes** where IL is entrapped in the membrane structure (Rynkowska, 2018);

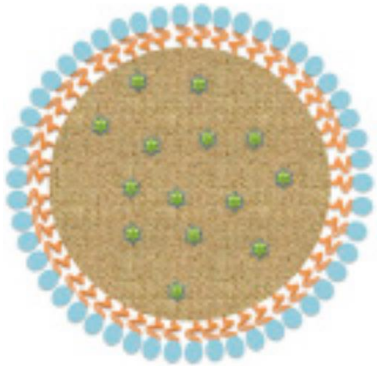
ILs grafts on stainless steel surface (Pang, 2015), microneedle **patches** for the transdermal delivery of therapeutics (T. Zhang, 2020), experimental orthodontic **adhesives** (He, 2021) and IL use on **surfactants** (Chauhan, 2017, Ghosh, 2021) exhibited significant antibacterial effects;



IL tagged Schiff base ligands can form complexes with transition metal ions also as catalysts in several reactions. They have reported antibacterial activities (Uddin, 2020),(Alkabli et al., 2020),(El-Sayed et al., 2021)

Results and discussion
- IL-assisted nanoparticles with antimicrobial properties:

- **Ag NPs** are often used for their inhibitory and bactericidal effect as ROS production cause direct damage of cell membrane (Anees Ahmad, 2020).
    - *IL functionalization* turns the NPs monodispersed to attach to bacterial cell surface and penetrate through the membrane, enhancing its activity (Patil, 2011).



PLGA nanoparticle

- **PLGA NPs** have high antibacterial activity under biofilms (C. Takahashi, 2019). Chitosan functionalization resulted in a ~20% decrease in the viable bacteria count and *incorporation of the IL*, resulted in an additional ~10% decrease compared to CS-PLGA NPs.

Results and discussion
- IL-assisted nanoparticles with antimicrobial properties:

- **zinc oxide (ZnO)** nanostructures with [BMIM][BF_4] exhibit higher zones of inhibition than with other ILs (Rajiv Gandhi, 2013) and ZnO NPs in ILs shows superior antibacterial efficacy in comparison with individually NPs or ILs (Aditya, 2018);

- **magnesium oxide (MgO)** NPs increase of bacterial sensitivity (Borkowski, 2019);

- presence of ILs in **silica oxide (SiO)** NPs caused the adsorption of bacteria onto the surface of NPs agglomerates (Borkowski, 2019);

- IL assisted green synthesis of rare earth elements **ytterbium oxide (Yb_2O_3)** NPs (Muthulakshmi & Sundrarajan, 2020), **samarium oxide (Sm_2O_3)** NPs (Muthulakshmi, 2020) and **neodymium oxide (Nd_2O_3)** NPs (Sundrarajan & Muthulakshmi, 2021) showed positive biological activities in antibacterial, anti-oxidant, anti-cancer and anti-inflammatory studies;

Results and discussion
- Solid-phase organic salts:

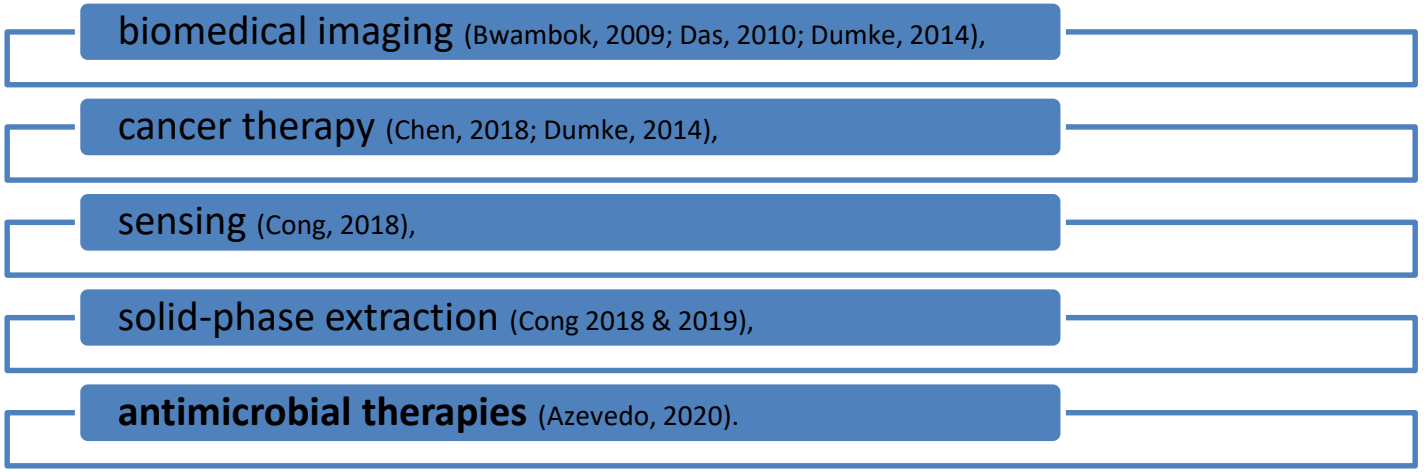
- ILs' applications can be limited by its defined thermal definition (100 °C) which lead to a limited number of counter-ions that produce nontoxic and functional ILs.

- **GUMBOS** redefine the useful limits of organic salts with same tunability as ILs, but with a wider range of applied cations and anions or melting points (Warner, 2014).

Results and discussion
- nanoGUMBOS:

- to date, there are already synthesized and characterized nanoGUMBOS with photothermal (Chen, 2019), photodynamic (Karam, 2015), luminescent (Dumke, 2010), and magnetic (Tesfai, 2009) properties.

- **tunable morphological, spectral and surface charge properties** as well as ***in vitro* behavior**, turns them promising candidates for several applications:



Conclusions

- AMR is considered one current major global public health problem
- most studied bacteria include Gram + *S. aureus*, *E. faecalis, E. faecium* and Gram - *P. aeruginosa*, *K. pneumoniae, A. baumannii,* with *E. coli* being also one of the most studied
- new antimicrobial targets and/or drugs are in great demand
- there are several synthesized ILs with reported antimicrobial properties
- other useful strategies include the use of ILs on composition or surface of biomaterials such as hydrogels, composites, polymers and NPs
- GUMBOS in nanotechnology form (nanoGUMBOS) are revolutionary as they for provide transport and antibacterial activity

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