Host immune modulation by antimicrobial drugs: current knowledge and implications for antimicrobial chemotherapy

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Giuliana Banche[a], Valeria Allizond[a], Narcisa Mandras[a], Vivian Tullio[a], Anna Maria Cuffini[a]

[a]Department of Public Health and Pediatrics; University of Torino, Via Santena 9, 10126 Turin, Italy

giuliana.banche@unito.it, valeria.allizond@unito.it, narcisa.mandras@unito.it, vivian.tullio@unito.it, annamaria.cuffini@unito.it

Corresponding author:

Giuliana Banche

Department of Public Health and Pediatrics

University of Torino

Via Santena 9, 10126 Turin, Italy

E-mail: giuliana.banche@unito.it

Phone: +390116705627

Fax: +390112365627

Abstract

It is known that antimicrobial agents possess several, beneficial, secondary properties which complement their primary antimicrobial activity like the immunomodulatory capacity that enforces host defense mechanisms or reduces host inflammatory response. In this review the current state of our recent research about the interaction between some antimicrobial agents and the immune system as complex pyramid of redundant cellular factors, humoral effectors and mediators against various microbial pathogens, will be presented and compared with recent literature data. The nature of such interactions is diverse and depends on the drug, the host immunological status and the microorganism. A more complete understanding of the host immune modulation by antimicrobial drugs may guide the selection of appropriate regimens for given clinical situations.

Chemical compounds studied in this article

Fosfomycin (PubChem CID:54331); Erythromycin (PubChem CID:12560); Tobramycin (PubChem CID:36294); Tiamphenicol (PubChem CID:27200); Caspofungin (PubChem CID:2826718).

Introduction

During the treatment of microbial infections with antimicrobials, an important interaction occurs between the drug administered and the host defenses to eradicate the invading pathogens; this phagocyte-drug interplay is a dynamic process *in vivo*: both direct and indirect effects may operate sequentially or simultaneously and the final outcome is often difficult to link to one or other phenomenon (**Figure 1**) [1]. However, antimicrobial agents, even microbicidal ones, may show limited efficacy when host defense mechanisms are defective due to numerous factors including profound and sustained neutropenia, medical device use, peritoneal dialysis or haemodialysis, organ transplant-associated immunosuppressive therapy, high dose corticosteroid treatment and HIV infection [1, 2]. Hence, over the past fifteen years scientists have tried to find drugs capable of enforcing host defenses [3] by modulating the primary phagocyte functions, the release of pro-inflammatory cytokines, the production of reactive oxygen species (ROS) or enzymatic pathways, whose products possess microbicidal properties [2,4]. On the other side of the coins, the host response to infection may be deleterious: pro-inflammatory cytokines and also secretory products of white blood cells are able to produce serious tissue damage. To try to cope with these effects, drugs that inhibit these effects have been searched for [3].

The current state of our recently research about the interaction of some antimicrobial agents on primary functions of human polymorphonuclear cells (PMNs), from both healthy subjects and immunonocompromised patients, against various pathogens is presented in this review. In particular, the influence of fosfomycin, erythromycin, tobramycin, tiamphenicol and caspofungin upon phagocytic ingestion and killing of intraPMNs Gram-positive and Gram-negative bacteria (*Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa*) and yeasts (*Candida albicans* and *C. glabrata*) is reported and compared with latest literature data about other professional phagocytic cell (i.e. monocytes, dendritic cells) activity and soluble factor/mediator production (i.e. cytokines and chemokines).

ANTIBACTERIAL AGENTS

Inhibitors of cell wall synthesis: phosphonic antibiotics

Fosfomycin

Fosfomycin (**Table 1**) [5-8] considered a first line drug in the eradication of urinary tract infections (UTIs), in addition to its antimicrobial activity, exhibits immunomodulatory effects on B-cell activation, lipopolysaccharide-stimulated monocytes and T-lymphocytes, by suppression of interleukin (IL)-2 production [9,10]. According to some authors fosfomycin affects the acute inflammatory cytokine response modulating the *in vivo* production of tumor necrosis factor (TNF)-

Ŭ, IL-1ɓ, and IL-6 [11], even though for others the concentrations of these cytokines are almost identical with and without fosfomycin [12]. Studies on fosfomycin effect on neutrophils highlighted enhanced phagocytic killing of invading pathogens, increased intracellular calcium concentrations, elevated extracellular reactive oxygen intermediate (ROI) production, and decreased chemotaxis, without affecting intracellular ROI production and chemokinesis [10ÅÅ13].

Our data [14] on the activity of fosfomycin tromethamine (FT) on the primary functions of PMNs from chronic haemodialysis patients (HDs) and renal transplant recipients (RTRs) compared to that of healthy subjects against an extended-spectrum beta-lactamase (ESBL) producing *E. coli* showed that FT, even at sub-inhibiting concentrations, is capable of inducing stimulation of the depressed phagocytic response of PMNs in these patients, restoring their primary functions *in vitro*. FT synergizes for bacterial killing with PMNs being able to kill *E. coli* when the phagocyte killing mechanisms fail because they are not sufficient in patients with renal dysfunctions. The clinical implication of our results can be emphasized by considering that these beneficial immunological properties of FT are observed at FT concentration much lower than those usually detected after FT administration in patients for treatment of uncomplicated UTIs (**Table 2**).

Inhibitors of protein synthesis: macrolides, aminoglycosides and phenicols

Erythromycin

Macrolides are used to treat soft tissue and respiratory tract infections (RTIs) [15,16]. Erythromycin, the progenitor of this class of antibiotics, together with clarithromycin, is widely used for the therapy of sinusitis and chronic obstructive pulmonary disease [16, 17]. In more recent years, azithromycin has been widely adopted as immunomodulatory agent for treatment of bronchiectasis and cystic fibrosis (CF) caused by mucoid *P. aeruginosa*, including resistant and biofilm producing strains [15-17]. There is an accumulating body of evidence over the last few years that macrolides (**Table 1**) [18Å20] are accumulated by PMNs, which, in turn, affect their active delivery to sites of microbial infection [18Å]. Part of the macrolide activity is not mediated through their traditional antimicrobial effect, but through their anti-inflammatory action, even at low doses [18Å20]. These effects include the ability to suppress the production and secretion of pro-inflammatory cytokines by neutrophils (IL-8, IL-1, TNF-α), reduce phagocyte infiltration into airways and secretion of mucus, prevent bacterial biofilm, modulate defensin and adhesion molecule expression [15,19], suppress nitric oxide production, promote inflammatory cell apoptosis, decrease the production of nuclear transcription factors and inhibit chloride and water secretion across the airway mucosa [16,18Å]. Macrolides may also interfere with signalling mechanisms initiated by activation of Toll-like receptors (TLRs): among them, erythromycin

treatment of monocyte-derived dendritic cells results in up-regulation of TLR2, down-regulation of TLR3, with no effect on TLR4 expression [18]. Erythromycin not only inhibits the production of IL-8 and suppresses the expression of IL-6 in human bronchial epithelial cells, but also modulates the lipoxygenase pathway of arachidonic acid metabolism in phagocytes reducing the leukotriene B4 concentrations in patients with chronic diseases resulting in improved pulmonary functions in these patients [19].

Significant antibiotic resistance has emerged throughout the world especially among those drugs commonly used to treat RTIs [21,22]. The alarming *in vitro* erythromycin resistance does not always correlate with poor clinical efficacy *in vivo* as standard susceptibility testing methods do not take into account the host defense mechanisms that play a key role during infection in preventing the triggering and spread of a bacterial infection process [23]. To highlight the potential erythromycin immunomodulatory properties related to different antibiotic resistance patterns in *Streptococcus* spp., we evaluated the influence of this macrolide on the PMN primary functions against erythromycin-susceptible (Ery-S) and erythromycin-resistant (Ery-R) *S. pyogenes* strains with different resistant phenotypes [22,24,25]. The results emphasise a significant high increase of intracellular killing by PMNs in the presence of erythromycin for all *S. pyogenes* strains tested. The most interesting data pertain to the ability of phagocytes to kill the intracellular Ery-R streptococci independently from their different levels of resistance to macrolide-lincosamide-streptogramin B antibiotics [24,25]. Since erythromycin even highly concentrated within phagocytes has a lower antimicrobial activity, compared with that of other antibiotics, owing to its intracellular instability in the acid medium, these data suggest that the enhanced intraPMN streptococcal killing detected is mainly attributable to PMN bactericidal systems that tightly co-operate with intracellular erythromycin in eradicating the Ery-R streptococci (**Table 2**) [24,25].

Tobramycin

Tobramycin [26,27] (**Table 1**) is widely used as an intravenous and inhaled therapy to treat *P. aeruginosa* lung infections. The improvement in lung function in treated CF patients suggests that inhaled tobramycin may have anti-inflammatory effects in addition to its bactericidal activity [26]. Gziut et al. [26] reported tobramycin anti-inflammatory properties *in vitro* related to its ability to bind copper, elevated in blood and sputum in CF: in fact, copper-tobramycin has intra and extracellular superoxide dismutase-like activity, with the potential to limit the expression of a wide range of adhesion molecules, inflammatory cytokines [28,29] and T-cell migration [28,30].

Tobramycin incorporated in solid lipid nanosphere (SLN) is better adsorbed by the gastrointestinal route and lasts longer in plasma with higher concentrations compared to administered tobramycin solution [31]. Furthermore tobramycin-loaded SLN is able to penetrate

bacterial barriers such as the outer membrane in Gram-negative bacteria. Our personal experiences documented that the use of tobramycin-loaded SLN may result in both great amount of intraPMNs antibiotic concentration and in a superior bactericidal activity towards intracellular *P. aeruginosa* in comparison with free non-SLN incorporated tobramycin [1Å31]. In fact, taking advantage of this delivery system, tobramycin, once intracellular, being able to remain microbiologically active, modulates the phagocyte bactericidal mechanisms by synergizing with them in eradicating intracellular bacteria. Our data suggest that tobramycin-loaded SLN is essential to obtain a superior therapeutic effect, dramatically by-passing the inefficiency in treating intracellular pseudomonal infections, that nowadays remain difficult and controversial to manage, particularly in severely ill patients (**Table 2**) [1Å27].

Thiamphenicol

The increased clinical isolation of macrolide-resistant strains has directed attention in recent years to alternative drugs, such as phenicols [32] (**Table 1**). However, studies on the host immune modulation by these antimicrobials are actually scanty. The available literature data regard chloramphenicol and florfenicol, a fluorinated synthetic analog of thiamphenicol, although largely used in clinical veterinary. Paape and Miller [33] tested the effects of chloramphenicol, thiamphenicol and florfenicol on bovine neutrophil phagocytosis and morphology indicating that neither florfenicol nor thiamphenicol alters neutrophils functions but their morphology. Zhang et al. [34] reported that florfenicol reduces TNF and IL-6 production by murine macrophages with little effect on IL-1β and IL-10 secretion. Páez et al. [35] only showed that chloramphenicol enables the oxidative stress response of human neutrophils and increases the ROS production. Recently in a retrospective study analysis of the activity of thiamphenicol glycinate (TG) administered to oncological patients affected by RTIs, Macchi et al. [36] showed that TG administered alone or in association with other antibiotics is globally effective in more than 95% of immunologically compromised patients.

Our data on the action of thiamphenicol (TAP) on human granulocyte functions against *S. pyogenes* [37] highlighted a synergistic activity between TAP and PMNs resulted in both phagocytosis and intracellular killing increased with time. It follows that TAP is efficient in the treatment of *S. pyogenes* RTIs due to its ability to act both directly against the pathogen and to boost bacterial intracellular killing in PMNs. These immunomodulating properties along with its broad spectrum, excellent pharmacokinetics, no haematological side-effects and good tolerability make TAP suitable for the treatment of RTIs especially in immunocompromised patients, such as oncological ones, with defective defense mechanisms (**Table 2**).

ANTIFUNGAL AGENTS

Inhibitors of beta-(1,3)-D-glucan synthase: echinocandins

Caspofungin

Under normal conditions, in fungi β-glucan epitopes are masked by other cell wall constituents, rendering them undetectable by host immune cells [2]. Echinocandins (**Table 1**) [38,39] have been recently found to have immune-stimulatory properties: at sub-inhibitory concentrations they may indirectly affect phagocyte activities through an alteration of fungal morphology that leads to an increased pathogen susceptibility to PMN actions. Following cellular uptake and intracellular accumulation, echinocandins modify immune responses with the release of pro-inflammatory or anti-inflammatory cytokines and affect production of ROS or enzymatic pathways, whose products possess antifungal properties [41]. Among echinocandins, caspofungin induces increases in dectin-1-mediated release of TNF-Ŭand chemokine ligand-2 by macrophages and augments antihyphal activity of PMNs against *Aspergillus* spp. and non-*Aspergillus* spp. [40]. Caspofungin significantly influences the oxidative burst metabolism and improves intracellular killing rates of *C. albicans*, but has no effect on phagocytosis; furthermore caspofungin decreases the cytokine (TNF-α and IL-1β) and chemokine release from activated monocytes improving host survival [41,42]. In addition, considering the critical role of TLRs in the immune system response, caspofungin detects the widest ability to allow *C. albicans* and *A. fumigatus* to stimulate TLR up-regulation on PMNs, specifically TLRs 4, 9 by *C. albicans* and TLR2 by *A. fumigatus*. TLRs stimulation on neutrophils can achieve reduction in chemotaxis, priming of superoxide generation, increase of phagocytosis and production of a number of cytokines and chemokines [43]. The only contradictory results are from van Asbeck et al [38] who observed that *C. parapsilosis*-caspofungin treatment alters the PMN capacity to phagocytose and delays killing of the yeast cells.

Our results [39,44-46] confirm the literature data [41,47] according to which caspofungin (CAS) has immune-enhancing properties that influence human PMNs from both immmunocompetent and immunocompromised subjects against yeasts. In detail, CAS at various concentrations displays a positive interaction with healthy subject PMNs on intracellular killing activity through a direct action exerted on either *C. albicans* or PMNs [39]. The most interesting result pertains CAS synergistic effect even on PMNs harvested from patients with renal dysfunction, such RTRs and HDs: the addition of CAS to RTR and HD-PMNs potentiates the intracellular fungal killing rates against both *C. albicans* [44,45] and a multidrug resistant (MDR) *C. glabrata* [46], achieving values similar to those observed for healthy subjects. These data underline the CAS role in the total restoration of the impaired PMN functions in

immunocompromised patients not only against susceptible yeast cells, but even against the MDR ones (**Table 3**).

Conclusions

As part of the immune system, a complex of redundant cellular factors, humoral effectors and mediators against pathogens, phagocytes, particularly neutrophils, are the central players in host resistance and the impairment of their functions is an important predisposing risk factor for the development of life threatening bacterial and fungal infections. Major advances have been achieved in understanding the immune enhancing properties of mainstream antibacterial and antifungal agents on host immune cells. All the data reported in this review support the conclusion that antimicrobial agents may exert other effects other than the antimicrobials such as either reinforcement of host defense mechanisms or dampening the host inflammatory response. The assessment of the host immune modulation by antimicrobial agents may therefore guide the selection of appropriate therapeutic regimens for given clinical scenarios and the creative use of the immunomodulatory properties of available antimicrobials may present a practical alternative especially when treating patients with defined immunological dysfunction, due to various factors [1Å,2].

Acknowledgments

We do apologize for not citing all the publications on this field due either to the ñmultifacetedò aspects or space limitation.

Conflict of interest statement

ñThe authors declare no conflicts of interestò

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Papers of particular interest, published within the period of review, have been highlighted as:

Åof special interest

ÅÅof outstanding interest

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Caption to Figure 1

Figure 1. A) Interaction between pathogens and polymorphonuclear cells (PMNs); **B**) Effect of antimicrobial agents on this interaction.

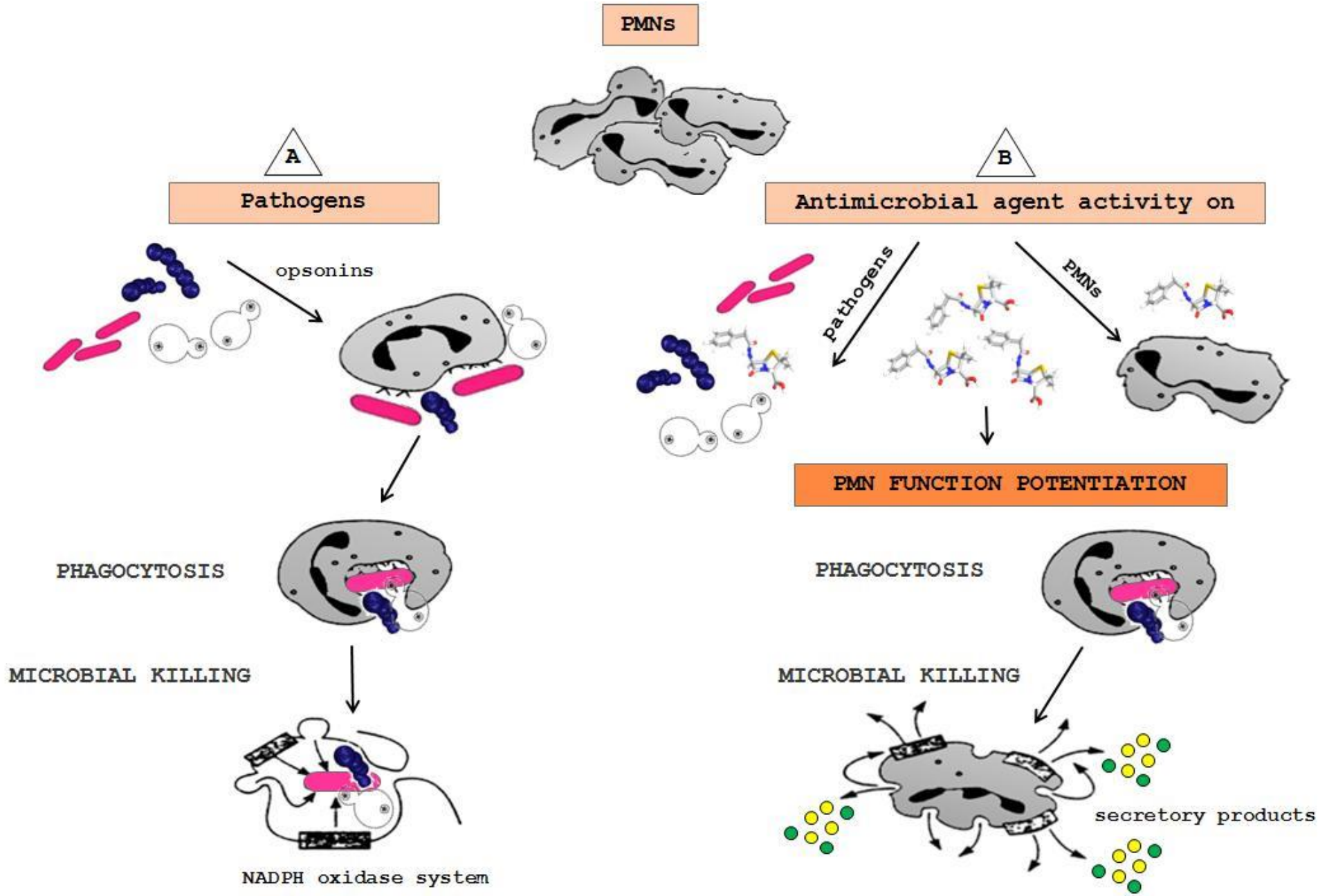
PMNs
A
Pathogens
opsonins
PHAGOCYTOSIS
MICROBIAL KILLING
NADPH oxidase system
B
Antimicrobial agent activity on
pathogens
PMNs
PMN FUNCTION POTENTIATION
PHAGOCYTOSIS
MICROBIAL KILLING
secretory products

Table 1. Characteristics of the antimicrobial agents tested.

ANTIBACTERIAL AGENTS		mode of action	spectrum of action	references
phosphonic antibiotics	fosfomycin	inhibition of the initial peptidoglycan synthesis process	wide antibacterial spectrum against Gram-positive, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci, and against a large number of Gram-negative pathogens, commonly isolated in urinary tract infections including extended-spectrum beta-lactamase *Escherichia coli*	[5-8]
macrolides	erythromycin	binding to P site of 50S ribosomal subunit by blocking bacterial protein synthesis steps	*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Mycoplasma* spp., *Legionella* spp. commonly isolated from respiratory infections, and *Staphylococcus* spp.	[15,18Å20]
aminoglycosides	tobramycin	irreversible binding to 16S r-RNA, on the 30S ribosomal subunit, inhibiting bacterial protein synthesis	broad antibacterial spectrum against Gram-negative nosocomial infections, severe systemic infections, chronic pulmonary infections sustained by *Pseudomonas aeruginosa*, and against Gram-positives, except *Streptococcus* spp. and *Enterococcus* spp.	[26,27]
phenicols	thiamphenicol	binding to 50S subunit inhibiting elongation step of protein synthesis	excellent activity against both Gram-negative and Gram-positive bacteria isolated from respiratory tract infections, prostatitis and sexually transmitted diseases. Its spectrum covers *Chlamydophila pneumoniae, Moraxella catarrhalis, Haemophilus influenzae, S. pneumoniae* and *S. pyogenes*, including ɓ-lactamase producing isolates, and anaerobes.	[32]
ANTIFUNGAL AGENTS		**mode of action**	**spectrum of action**	**references**
echinocandins	caspofungin	inhibition of fungal β-1,3-glucan and β-1,6-glucan synthesis preventing fungal growth	excellent antifungal activity against *Candida* spp., *Aspergillus* spp., *Histoplasma* spp., *Blastomyces* spp. and *Coccidioides* spp.	[38,39]

Table 2. Host immune modulation by antibacterial agents on PMN primary functions against bacterial pathogens.

ANTIBACTERIAL AGENTS					drug effects on functional PMN activities						
							drug pre-treated bacteria		drug pre-treated PMNs		
	tested bacterial strains (10^7cfu/ml)	**MIC**	**tested drug concentration (μg/ml)**	**PMNs (10^6 cells/ml)**	**phagocytosis**	**intracellular killing**	**phagocytosis**	**intracellular killing**	**phagocytosis**	**intracellular killing**	**references**
fosfomycin	ESBL producing *Escherichia coli* ATCC 35218	8 μg/ml	1/16 x MIC	HSs RTRs HDs	potentiation	potentiation	indifference	potentiation	indifference	potentiation	[14]
erythromycin	*Streptococcus pyogenes* Ery-S	0.25μg/ml	MIC ¼ x MIC	HSs	indifference	potentiation	-	-	-	-	[24,25]
	Streptococcus pyogenes Ery-R	8 μg/ml ($iMLS_B$-C) 16 μg/ml (M phenotype) 128 μg/ml ($cMLS_B$, $iMLS_B$-A, $iMLS_B$-B)	MIC ¼ x MIC	HSs	indifference	potentiation	-	-	-	-	
tobramycin	*Pseudomonas aeruginosa*	2 μg/ml	MIC	HSs	potentiation	potentiation	potentiation	potentiation	indifference	potentiation	unpublished data [1]
thiamphenicol	*Streptococcus pyogenes*	8 μg/ml	½ x MIC	HSs	potentiation	potentiation	indifference	potentiation	indifference	potentiation	[37]

Legend: cfu (colony forming unit); MIC (minimal inhibitory concentration); PMN (polymorphonuclear cell); ESBL (extended-spectrum beta-lactamase) Ery-S (erythromycin-susceptible); Ery-R (erythromycin-resistant); MLS_B (macrolide lincosamide streptogramin B) resistance: $cMLS_B$ (constitutive phenotype) or $iMLS_B$ (inducible phenotype) with $iMLS_B$-A (subtype A), $iMLS_B$-B (subtype B) or $iMLS_B$-C (subtype C); HSs (healthy subjects); RTRs (renal transplant recipients); HDs (haemodialyzed patients) .

Table 3. Host immune modulation by antibacterial agents on PMN primary functions against yeasts.

ANTIFUNGAL AGENT					drug effects on functional PMN activities						
							drug pre-treated yeasts		drug pre-treated PMNs		
	tested fungal strains (10^6 cfu/ml)	**MIC**	**tested drug concentration (μg/ml)**	**PMNs (10^6 cells/ml)**	**phagocytosis**	**intracellular killing**	**phagocytosis**	**intracellular killing**	**phagocytosis**	**intracellular killing**	**references**
caspofungin	*Candida albicans*	2 μg/ml	MIC 3.2 μg/ml (peak serum level) 4 x MIC	HSs	indifference	potentiation	-	potentiation	-	potentiation	[39]
			MIC ½ x MIC ¼ x MIC	HSs RTRs HDs	indifference	potentiation	-	-	-	-	[44,45]
	Candida glabrata MDR	4 μg/ml	MIC ½ x MIC	HSs RTRs HDs	indifference	potentiation	-	-	-	-	[46]

Legend: cfu (colony forming unit); MIC (minimal inhibitory concentration); PMN (polymorphonuclear cell); MDR (multidrug resistant); HSs (healthy subjects); RTRs (renal transplant recipients); HDs (haemodialyzed patients)