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Original article

Antibacterial and antibiofilm activity of Arixib: A nonsteroidal anti-inflammatory drug

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SUMMARY

Antibiotic resistance is a growing threat to global health, emphasizing the urgent need for new therapeutic strategies. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as arixib, initially developed for their anti-inflammatory properties, are increasingly being explored for their potential antibacterial effects. This study aimed to evaluate the antibacterial activity of arixib against six bacterial strains, including four reference strains and two multidrug-resistant clinical strains, as well as its effects on biofilm formation and dispersal.

The experimental protocol involved applying controlled dilutions of arixib to bacterial cultures grown in 96-well microtiter plates, followed by biofilm staining with crystal violet and quantification using spectrophotometry. The results revealed a variable antibacterial activity of arixib, depending on the tested strains, with significant inhibition observed at specific concentrations. Notably, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus exhibited complete bacterial inhibition at 0.5 mg/ml, whereas Klebsiella pneumoniae displayed greater resistance, requiring higher concentrations.

Regarding biofilm formation, arixib demonstrated concentration-dependent inhibitory effects, with complete absence of biofilm at higher concentrations for

most strains tested. However, lower concentrations resulted in partial resistance, particularly in resistant clinical strains such as E. NDM-1. In addition, arixib exhibited a notable dispersal effect, significantly reducing biofilm mass in a concentration-dependent manner.

Introduction

Antibiotic resistance remains one of the most pressing public health challenges of the 21st century. Since Alexander Fleming's discovery of penicillin in 1928, antibiotics have revolutionized medicine by offering a powerful solution against bacterial infections. However, the widespread and often inappropriate use of these drugs has led to the emergence of resistant bacterial strains, severely limiting the effectiveness of conventional treatments (Ramanan et al., 2013; Ventola, 2015; Munita, 2016; Hrvatin, 2017; Landers, 2012). According to the World Health Organization (WHO), infections caused by antibiotic-resistant bacteria are responsible for hundreds of thousands of deaths worldwide each year (WHO, 2021; O'Neill, 2016).

Beyond antibiotic resistance, the formation of bacterial biofilms presents another major challenge in infection management (Mah & O'Toole, 2001). Biofilms are communities of bacteria embedded in a self-produced extracellular matrix, adhering to surfaces either biotic or abiotic (Hall-Stoodley et al., 2004 ; O'Toole et al., 2000). This protective structure not only shields bacteria from antimicrobial agents but also from the host's immune defenses, making infections more chronic and prone to recurrence (Anmol et al., 2004). Biofilms are responsible for around 80% of chronic human infections, including lung infections in cystic fibrosis patients, medical device-associated infections, and chronic wound infections (Donlan, 2001 ; Costerton et al., 1999).

Biofilm formation is intrinsically linked to antibiotic resistance. Bacteria within biofilms exhibit heightened resistance to treatment compared to their free-floating (planktonic) counterparts. This increased resistance is due to several factors, such as limited antibiotic diffusion through the biofilm matrix, the expression of resistance genes, and the altered physiological state of bacterial cells within biofilms (Mah and O'Toole, 2001). These characteristics make biofilm-associated infections particularly difficult to eradicate, even with high-dose antibiotic treatments.

Research is increasingly focused on understanding the mechanisms driving biofilm formation and antibiotic resistance, as well as developing novel strategies to combat these phenomena. Promising approaches include biofilm-disrupting agents, targeted antibiotic delivery systems, and the development of new antimicrobial compounds capable of overcoming resistance barriers (Koo et al., 2017).

In summary, antibiotic resistance and biofilm formation are closely intertwined challenges that significantly hinder the treatment of bacterial infections. A deeper understanding of these mechanisms is crucial for the development of innovative and effective therapeutic strategies.

Recently, there has been growing interest in repurposing drugs originally developed for well-defined conditionssuch as diabetes, cancer, inflammation, and psychological disorders for their potential antibacterial properties. Among these, nonsteroidal anti-inflammatory drugs (NSAIDs), traditionally used to reduce inflammation and fever, have garnered attention for their possible antimicrobial

activities.

This study aims to evaluate the antibacterial potential of arixib, a particular NSAID. Additionally, we have developed a method to quantify biofilm eradication, accounting for factors such as bacterial strain incubation time and the concentration of the tested compound

. **Materials and Methods**

1. Antibacterial activity:

The antibacterial activity of Arixib was tested against four bacterial strains (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *S. aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853,) and two multidrug resistant clinical strains. The inoculum suspension was obtained by taking colonies from 24 h cultures. The colonies were suspended in sterile 0.9% aqueous solution of NaCl. The density was adjusted to the turbidity of a 0.5 McFarland Standard (10^8 colony-forming unit [CFU]/ml).

The molecule to be tested was resuspended in DMSO at a concentration of 2 mg/ml.

2. Determination of minimum inhibitory and minimum bactericidal concentrations

The MIC is the minimal concentration of the antimicrobial agent which can inhibit visible growth of the microorganisms in 24h. It was determined by the broth micro-dilution technique using 96-well microtiter plates (Bazargani and Rohloff 2016). Briefly, the bacterial suspension was prepared from an overnight culture and adjusted to a concentration of 10^8 CFU/mL in Muller Hinton Broth (MHB). Serial dilutions of the studied molecule ranging from 1 to 0.06 mg/mL were processed on MHB with dimethylsulfoxide (DMSO). MHB added with the inoculum and MHB with DMSO were used as positive and negative controls, respectively. The plates were incubated at 37 °C for 24 h.

The MBC is the lowest concentration of the antimicrobial agent capable of reducing 99.9% of the initial bacterial population after 24 hours. It was determinted as reported by Moreira et al. 2005

Since the molecule tested is colored, this makes it difficult to visualize the turbidity resulting from bacterial growth and to avoid any error in the estimation of the MIC, an aliquot of 20 µL of the bacterial culture on the different concentrations of the tested molecule is spread on Luria Bertani (LB) agar medium, a rich medium allowing the visualization of bacterial growth.

3. Biofilm Formation assay

Biofilm formation was assessed using the microtiter plate method with crystal violet staining, as described by O'Toole (1999). Briefly, 200 µL of diluted bacterial culture was added to each well of a sterile 96-well flat-bottom polystyrene plate and incubated at 37°C for 24 hours under static conditions. After incubation, non-adherent cells were removed by washing the wells three times with phosphate-buffered saline (PBS, pH 7.4). The biofilm biomass was then stained with 0.1% (w/v) crystal violet for 20 minutes at room temperature.

4. Biofilm Inhibition test

To evaluate the antibiofilm effect of arixib, the microplates used for antibacterial tests were washed with distilled water to remove non adherant cells, dried, and then stained with 0,1% of crystal violet for 20 minutes. After rinsing and drying, a qualitative observation of the biofilms was performed.

To quantify the biofilms, 400 µl of 96% ethanol was added to the wells followed by measurement of the optical

density (OD) at 600 nm.

A blank of 2000 µl of ethanol served as a reference for all measurements, thus allowing the effect of arixib on biofilm formation to be quantitatively evaluated. Each experiment was performed in triplicate.

Biofilm inhibition rates of initial cell adhesion were calculated for each arixib concentrations as follows **(**Ceri et al. 1999).

% Inhibition = (OD PC- OD TC / OD PC) X100

OD TC : Optical Density of culture incubated with the Tested Concentration

OD PC : Optical Density of Positif Control

5. Biofilm Eradiation

During biofilm formation and after incubation times, we observed a non-homogeneity of biofilms in the wells.

Based on the work reported by De Vincenti et al. 2018, we developed an original way to calculate the percentage of biofilm dispersion in each well without involving the value of the positive control that is generally used in the reported works (Laktib et al., 2024). To study the dispersive effect of arixib on biofilms, six bacterial cultures in microplates were incubated at 37°C at different time intervals: 3, 6, 9, 15, 18 and 24 hours. Each plate contained bacteria in the first wells, followed by two negative controls (LB) and (DMSO). After incubation, the biofilms were stained with crystal violet and then treated with serial dilutions of arixib, starting with 1 mg/ml. The plates were incubated for 24 hours at 37 °C. After incubation, the OD of each well was recorded: this OD corresponds to the number of bacteria eliminated from the biofilm by the concentration of the molecule tested (supernatant OD). Then the plate is washed with distilled water and then treated with 400 µl of 96% ethanol and the OD of the wells is read : This OD corresponds to the number of bacteria that remained in the biofilm after treatment with the concentrations of the molecule tested (OD of the biofilm remaining attached). The biofilm dispersion rate is given by the formula below:

$$\text{Eradication rate} = \frac{(\text{OD of the supernatant with detached bacteria})}{\text{OD of Biofilm remaining attached} + \text{OD of the supermanant}} \times 100$$

OD_BF : O.D of the remaining sessile bacteria after action of the molecule (Biofilm bacteria)

OD Sup : O.D of the planktonic bacteria detached by action of the tested molecule (Bacteria contained in the supernatant)

Results

1. Antibacterial activity of Arixib

Except for *K. pneumoniae*, which shows high resistance to Arixib, the other reference strains exhibit identical MICs of 0.25 µg/ml. However, the clinical strains display an MIC of 0.5 µg/ml, almost double that of the reference strains (Table 1). The results obtained show that Arixib exhibits variable antibacterial activity depending on the strains tested. For *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus*, complete inhibition was observed at a concentration of 0.25 mg/ml, while *Klebsiella pneumoniae* demonstrated greater resistance to Arixib, up to a concentration of 3 mg/ml. Clinical strains with multidrug resistance profiles (E. CTXM9 and E.NDM-1) showed inhibition at 0.5 mg/ml, which is twice that of the reference strains. It is noteworthy that these strains harbor resistance genes carried by high-molecular-weight plasmids (Barguigua et al., 2014), suggesting that these plasmid-type genetic elements may contribute to the increase in the inhibitory concentration of the tested molecule. The absence of bacterial growth in the negative controls (MHB alone and DMSO alone) validates the methodology, indicating that the observed results are due

to the effects of Arixib. These findings highlight Arixib's potential efficacy against certain strains while emphasizing the need for further research to understand and overcome the resistance observed in some bacteria and explore the mechanisms of action of the studied molecule.

Table 1 : Effect of Arixib on bacterial growth (Qualitative observation)

Conc. of compound (mg/ml)	Bacteria (CMI mg/ ml)					
	E. coli	*K.pneumoniae*	*S. aureus*	*P. aeruginosa*	*E.*CTXM9	*E.*NDM1
5						
3		3				
2						
1						
0.5					0.5	0.5
0.25	0.25		0.25	0.25		

Bactericidal effet of arixib

The results (Table 2) show that arixib has a significant bactericidal effect on several bacterial strains at different concentrations. For *Escherichia coli*, wells 1 (1 mg/ml) and 2 (0.5 mg/ml) show notable bactericidal percentages with values of 97% and 90.74% respectively. A similar trend is observed for *Pseudomonas aeruginosa*, with bactericidal percentages of 87% in the same wells. *Staphylococcus aureus* shows a progressive decrease in bactericidal percentage, from 74% to 68.54% in the first wells. Modified strains, such as *E.* CTXM9 also show notable bactericidal percentages, particularly in wells 1 and 2, indicating high susceptibility to arixib. Negative controls showed no inhibition, confirming the specificity of the effect of arixib. These results suggest that arixib has a varied antibacterial potential depending on the strains, with notable efficacy against commonly resistant bacteria such as *E.NDM-1* and *E. CTXM9*, although some strains such as *Klebsiella pneumoniae* show higher resistance.

2. Biofilm Formation

Analysis of the results shows (Table 3) that Arixib inhibits biofilm formation in a concentration-dependent manner. For *E. coli*, *E.* CTXM9, *P. aeruginosa*, *S.aureus*, *K.pneumoniae*, and *E.* NDM-1, no biofilm formation was observed in the first three wells (1 mg/ml, 0.5 mg/ml, and 0.25 mg/ml), while biofilm presence was detected from well 4 to well 10. No culture is observed in the negative controls. These results suggest that Arixib has significant potential to inhibit biofilm formation at specific concentrations, although its efficacy varies depending on the bacterial strains, highlighting the need for further research to optimize its use and understand the mechanisms of inhibition.

The results of the experiment (Table 4) show that arixib exerts a significant inhibitory activity on bacterial biofilm formation, which varies according to the concentrations tested. For *E. coli, P.aeruginosa a*nd *E. NDM-1*, the optical density (O.D.) values decrease noticeably with the increase in arixib concentration, indicating an increasing inhibition of biofilm formation. For instance, *E. coli* exhibits very

low O.D. values in wells containing high concentrations of arixib (wells 1 to 3), demonstrating strong inhibition of biofilm formation compared to wells with lower concentrations. Similarly, a significant reduction in O.D. is observed for *Pseudomonas* at high arixib concentrations (wells 1 to 3), indicating marked inhibition of biofilm formation. However, for NDM-1, although arixib shows inhibitory activity (reduction of O.D. in wells 1 to 3), this inhibition appears to decrease at lower concentrations. The percentage inhibition calculations, obtained by subtracting the O.D. of the positive control from the values of the wells containing arixib, confirm this observation by showing higher percentages of inhibition at higher concentrations of arixib for *E. coli* and *P. aeruginosa*. The negative controls showed no biofilm formation, validating the absence of external factors such as DMSO alone. These results suggest that arixib presents promising potential as a biofilm-inhibiting agent, particularly at appropriate concentrations, which deserve further exploration to optimize its potential efficacy in clinical settings. Its effectiveness decreases with the reduction of arixib concentration.

Table 2 : Quantification of bacterial growth and inhibition rate

		Arixib molecule (mg/ml)					Control (+)
		1	0,5	0,25	0,125	0,0625	LB + BACT
E. coli	OD_sup	0,04	0,125	0,35	0,46	0,8	1,35
	% BC	97	90,74	74,7	65,55	40,74	
K. pneumoniae	OD_sup	0,28	0,55	0,70	0,73	0,74	1,8
	% BC	84,44	69,44	61,11	59,44	58,88	
S. aureus	OD_sup	0,275	0,335	0,545	0,695	0,73	1 ,065
	% BC	74	68,54	48,82	34,74	31,45	
P. aeruginosa	OD_sup	0,19	0,20	0,365	0,605	0,95	1,45
	% BC	87	86	74,82	58,27	34,48	
E. coli CTXM9	OD_sup	0,1	0,16	0,2	0,21	0,26	0,865
	% BC	88,43	81,5	77	76	70	
E. coli NDM-1	OD_sup	0,185	0,2	0,245	0,345	0,435	1,83
	% BC	90	89	86,61	81,14	76	

Table 3 : Qualitative observation of biofilm formation

	1 mg/ml	0,5 mg/ml	0.25 mg/ml	0,125 mg/ml	0,0625 mg/ml
E. coli	-	-	-	+	+
K. pneumoniae	-	-	-	+	+
S. aureus	-	-	-	+	+
P. aeruginosa	-	-	-	+	+
*E.*CTXM9	-	-	-	+	+
E .NDM-1	-	-	-	+	+

Table 4 : Quantification of biofilms after ethanol treatment

		Arixib molecule (mg/ml)					Contrôle (+)
		1	0,5	0,25	0,125	0,0625	LB + BACT
E. coli	OD_BF	0,19	0,20	0,33	0,35	0,67	1,135
	% inhibition	83	82	71	69	41	
K. pneumoniae	OD_BF	0,12	0,17	0,48	0,56	0,57	0,8
	%inhibition	85	78	40	30	28	
S. aureus	OD_BF	0,31	0,32	0,33	0,61	0,89	1,07
	% inhibition	71	70	69	43	16	
P. aeruginosa	OD_BF	0,1	0,17	0,58	0,89	0,97	2,04
	% inhibition	95	91	71	56	52	
E. coli CTXM9	OD_BF	0,16	0,18	0,21	0,31	0,82	1
	% inhibition	83	82	79	69	18	
E. coli NDM-1	OD_BF	0,02	0,355	0,55	0,61	0,66	0,91
	% inhibition	98	61	39	33	27	

Eradication of the formed biofilms

During the formation of biofilms after their incubation times, we observed a non-homogeneity of the biofilms in the wells, inspired by the work published by De Vincenti et al., 2018, we established an original way to calculate the percentage of dispersion.

The results of this study (Table 5) reveal that arixib exhibits variable efficacy in reducing bacterial biofilms depending on the strain studied, the decreasing administered concentration, and the incubation time. On average, arixib demonstrated significant biofilm eradication capacity for all strains tested, with eradication percentages ranging from 20% to 80%. Strains such as *E. coli* and *K. pneumoniae* showed a favorable response to specific concentrations of arixib, reaching up to 75% eradication, The results also indicate an increase in the eradication efficacy of arixib with decreasing concentration, suggesting a promising potential of this

agent for the treatment of biofilm infections. These observations highlight the importance of considering the diversity of microbial responses in the development of targeted therapeutic approaches against bacterial biofilms.

Table 5 : Quantification of biofilm eradication and dispersion after arixib treatment

		'Arixib molecule (mg/ml)					
		1	0,5	0,25	0,125	0,0625	0,03125
E. coli	OD_sup	0,285	0,9	0,335	1,35	1,43	0,99
	OD_BF	0,855	1,86	0,465	1,42	1,33	0,39
	% eradic	25	33	42	49	52	72
Kpneumoniae	OD_sup	0,285	0,33	0,39	0,92	1,525	1,525
	OD_BF	0,61	0,585	0,54	0,475	0,475	0,45
	% eradic	32	36	42	66	76	77
P.aeruginosa	OD_sup	0,825	0,505	0,52	2,185	0,715	0,88
	OD_BF	2,485	1,065	1	3,465	0,92	0,9
	% eradic	25	32	34	39	44	49
S.aureus	OD_sup	0,515	0,6	0,715	0,1	1,135	0,2
	OD_BF	2,095	2,255	1,98	0,2	1,96	0,3
	% eradic	20	21	27	33	37	40
E.CTXM9	OD_sup	0,53	0,325	0,57	0,69	0,575	0,25
	OD_BF	1,575	0,52	0,835	0,895	0,585	0,20
	% eradic	25	39	40	44	49	55
E. NDM-1	OD_sup	0,125	0,71	0,555	0,495	1,57	0,475
	OD_BF	0,325	1,545	1,03	1,665	1,405	0,4
	% eradic	28	31	35	47	53	54

Discussion

The results show that arixib exhibits significant inhibition of bacterial growth for *E. coli* and *P. aeruginosa* at concentrations of 1 mg/ml and 0.5 mg/ml. This observation is consistent with previous studies on the antibacterial effects of non-steroidal anti-inflammatory drugs (NSAIDs), as demonstrated with ibuprofen and aspirin, which can inhibit the bacterial growth of several strains such as *E. coli* and *P. aeruginosa* by disrupting fatty acid synthesis **(**Kumar et al.,2015)**.** *K. pneumoniae* showed strong resistance to arixib in these experiments, with growth observed at concentrations of 3 mg/ml. This may be due to the natural resistance of certain strains. Indeed, the reference strain used in this work is the strain of *K. pneumoniae* ATCC 13883 which has an intrinsic resistance to penicillin G and other natural penicillins, aminopenicillins and first-generation cephalosporins. Macrolides and lincosamides are also ineffective against

this strain. The presence of genes involved in resistance to these antibiotics could contribute to the ineffectiveness of arixib at low concentrations.

For *E.* CTXM9 and E. NDM-1, inhibition was observed only at the highest concentration of arixib (1 mg/ml), suggesting resistance mechanisms that allow them to tolerate lower concentrations of the drug. This can be explained by the presence of resistance genes such as CTXM9 and NDM-1, which confer increased resistance to bacterial strains by producing enzymes that inactivate a wide range of antibiotics, including NSAIDs **(**Bonnet, R. 2004, Mah, TF and O'Toole, G A. 2001)**.**

The results of our study show that arixib significantly inhibits biofilm formation at high concentrations (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml) for several bacterial strains, including *E. coli*, *P. aeruginosa*, and *E.*NDM1. For example, O.D. values for *E. coli* are very low in the first three wells, indicating almost complete inhibition of biofilm formation. This observation is supported by previous studies that have shown that anti-inflammatory compounds can significantly reduce biofilm formation by disrupting the cellular signaling necessary for their development **(**Kumar et al.,2015)**.**

The results indicate that arixib has a variable but significant capacity to disperse bacterial biofilms, with increased efficiency observed at lower concentrations, suggesting a potential for biofilm matrix disruption at sub-inhibitory levels. This observation is consistent with the theory that reduced doses can optimize anti-biofilm action by minimizing resistance effects and maximizing diffusion within the biofilm. This is similar to a study exploring the use of *Bacillus* probiotics, which showed an ability to disperse pathogenic biofilms at sub-inhibitory concentrations by interfering with bacterial communication signals, reducing resistance and promoting pathogen elimination **(**Piewngam et al., 2018)

Conclusion

This research aimed to evaluate the antibacterial and anti-biofilm properties of arixib, a goal achieved through a series of in-depth experiments. The results revealed variable arixib activity against different bacterial strains: effective growth inhibition for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* at concentrations of 1 mg/ml, contrasting with notable resistance from *Klebsiella pneumoniae*, which required a higher dose of 5 mg/ml. In terms of biofilm inhibition, arixib demonstrated notable efficacy down to 0.25 mg/ml for most strains. Additionally, arixib exhibited significant biofilm eradication capacity, with dispersion rates ranging between 20% and 80%, depending on the strain and administered concentration. These results highlight the promising potential of arixib as a multifunctional agent against bacterial infections, particularly in the treatment of resistant biofilms. However, this study has some limitations, notably the use of optical density-based methods to quantify biofilms, which could affect the accuracy of the results. Moreover, the study focused exclusively on arixib as a single agent, leaving room for future research on its efficacy in combination with other antimicrobial agents. For future perspectives, it would be beneficial to further explore the underlying mechanisms responsible for the observed variations in arixib activity among the different bacterial strains. Additionally, in vivo studies and clinical trials are necessary to fully evaluate the efficacy and safety of arixib in a clinical context, as well as its potential in combination with other therapies.

In conclusion, this study opens new perspectives for the use of arixib as a potential treatment for resistant bacterial infections and biofilms, offering significant opportunities to improve treatment strategies and clinical outcomes in the fight against bacterial infections.

Conflict of interest

None

References

- Anmol, S., Nidhi, V., Vivek, K., Pragati, A. and Vishnu, A. (2024) "Biofilm inhibition/eradication: exploring strategies and confronting challenges in combatting biofilm", Archives of Microbioogy, 14 ; 206 (5) :212.
- Barguigua, A., El Otmani, F., Lakbakbi El Yaagoubi,F., Talmi, M., Zerouali, K. and Timinouni , M. (2013) "First report of a Klebsiella pneumoniae strain coproducing NDM-1, VIM-1 and OXA-48 carbapenemases isolated in Morocco"APMIS. 121(7) :675- 677.
- Bazargani MM, Rohloff J (2016) "Antibiofilm activity of essential oils and plant extracts against Staphylococcus aureus and *Escherichia coli biofilms"* Food Control 61 :156–164.
- Bonnet, R. (2004) "Growing group of extended-spectrum β-lactamases: the CTX-M enzymes" Antimicrobial Agents and Chemotherapy, 48 (1), 1-14,
- Ceri, H., Olson, M. E., Stremick, C., Read, R. R., Morck, D. and Buret, A. (1999) "The Calgary Biofilm Device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms". Journal of Clinical Microbiology, 37 (6), 1771-1776.
- Costerton, J. W., Stewart, P. S., Greenberg, E. P. (1999) "Bacterial biofilms : a common cause of persistent infections" Science, 284(5418), 1318-1322.
- De Vincenti, L., Glasenapp, Y., Cattò, C., Villa, F., Cappitelli F. and Papenbrock J. (2018) "Hindering the formation and promoting the dispersion of medical biofilms : non-lethal effects of seagrass extracts" BMC Complementary and Alternative Medicine 18 (1) :168
- Donlan RM. (2001) "Biofilms and device-associated infections" Emerging Infectious Diseases. 7(2) :277-281
- Hall-Stoodley L, Costerton JW. and Stoodley P. (2004) "Bacterial biofilms : from the natural environment to infectious diseases" Nat Rev Microbiol. 2(2) :95-108
- Hirsch, A.C., Smith, S. and Gadikota, R.R. (2014). "Effect of non-steroidal anti-inflammatory drugs on biofilm formation and bacterial resistance" PLoS *ONE*, 9(5), e97325.
- Munita, JM. and Arias, CA. (2016) "Mechanisms of Antibiotic Resistance" Microbiology Spectrum. 4(2).
- Koo, H., Allan, R. N., Howlin, R. P., Stoodley, P. and Hall-Stoodley, L. (2017) "Targeting microbial biofilms : current and prospective therapeutic strategies" Nature Reviews Microbiology, 15(12), 740-755.
- Kumar, A., Dubey, K. K. and Singh, S. K. (2015) "NSAIDs inhibit bacterial growth and biofilm formation, and modulate microbial communities in polymicrobial infections" Antimicrobial Agents and Chemotherapy, 59(1), 429-437,
- Laktib, A., · Fayzi, L., El Megdar, S., El Kheloui R., Msanda, F., Cherifi, K., Hassi, M., Ait Alla,A., Mimouni, R. and Hamadi, F. (2024) "Inhibition and eradication effects of *Thymus leptobotrys* and *Thymus satureioïdes* essential oils against multidrug resistant Acinetobacter baumannii biofilms" Biologia, 79, 1003-1013
- Dai, l., Wu, Tq., Wang, ZH., Yuan R., Ding, Y., Zhang,WC., Chu, SP., Ju, Sq. and Yu. J. (2019) "Ibuprofen-mediated potential inhibition of biofilm development and quorum sensing in Pseudomonas aeruginosa. Life Sciences. 15 :237
- Mah, T. F. and O'Toole, G. A. (2001). "Mechanisms of biofilm resistance to antimicrobial agents" Trends in Microbiology, 9(1), 34-39.
- Mahdi, S.H. and Mahdi, A.S. (2020). "Ibuprofen as a potential antibiofilm agent" Journal of Infection and Chemotherapy, 26(10), 1092-1097.
- Moreira, MR., Ponce, AG., Del Valle, CE. and Roura SI (2005) "Inhibitory parameters of essential oils to reduce a foodborne pathogen" LWT - Food Science and Technology 38 : 565–570.
- O'Neill, J. (2016). "Tackling Drug-Resistant Infections Globally: Final Report and Recommendations." Review on Antimicrobial Resistance.
- O'Toole, G., Kaplan, H B. and Kolter R. (2000) "Biofilm formation as microbial development" Annual Review of Microbiology, 54 :49-79.
- Piewngam, P., Zheng, Y., Nguyen, T. H Dickey, SW., Joo HS., Villaruz , AE., Glose, KA., Fisher, E.L., Hunt, RL., Li, B., Chiou, J., Pharkjaksu, S., Khongthong, S., Cheung, G.YC., Kiratisin, P. and Otto, M. (2018) "Pathogen elimination by probiotic Bacillus via signalling interference" Nature, 562(7728), 532-537
- Laxminarayan , R., Duse, A., Wattal,C., Zaidi, AKM., Wertheim, HFL., Sumpradit, N., Vlieghe, E., Hara, GL., Gould, IM., Goossens, H., Greko, C., So, AD., Bigdeli,M., Tomson, G., Woodhouse,W., Ombaka, E., Peralta,AQ., Qamar, FN., Mir, F., Kariuki, S., Bhutta, ZA., Coates, A., Bergstrom, R., Wright, CD., Brown, ED. and Cars, O. (2013) "Antibiotic resistance-the need for global solutions" Lancet Infectious Diseases ;13(12) :1057-98.
- Landers, TF., Cohen, B., Wittum, TE. and Larson, EL. (2012) "Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential" Public Health Reports. 127(1) : 4–22.
- Hrvatin, V. (2017) "Combating antibiotic resistance : New drugs or alternative therapies?" Canadian Medical Association Journal 189 (37): E1199
- Ventola C L. (2015) "The antibiotic resistance crisis: part 1: causes and threats" Pharmacy and Therapeutics. 40(4) :277-283
- World Health Organization. (2021). "Global action plan on antimicrobial resistance" Geneva: World Health Organization