RESEARCH ARTICLE

The Role of Counter-Anions on Cationic Antimicrobial Agents

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Abstract
Even though a myriad of antimicrobial agents have been developed over the decades, resistant mechanisms continually break through, ultimately increasing our mortality and fears. Significant efforts have been made to evade these pathogenic resistances by developing novel antimicrobial agents which work on different targets. One possible, simple solution that has also been investigated has been to modify the counter-anion of cationic-based drugs. Although a multitude of studies have evaluated the efficacy of the active cationic agent, some have also explored the often-neglected influence of the counter-anion. Understanding the role of the counter-anions may provide new antimicrobial agents and an alternative approach to quell antimicrobial resistance. This review focuses on the various studies that have either directly or in-directly evaluated the role of the counter-anion on antimicrobial activities. Indeed, certain cationic-based agents display significant alternation in their activity when paired with the correct anion.

Keywords:
Antimicrobial Resistance; Counter-Anion; Minimum Inhibitory Concentration; Organic; Inorganic

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1. Introduction

Worldwide, infectious disease is one of the leading causes of the death annually.[1] From the first development of the antibiotic, penicillin to the new and more powerful antimicrobial agents of today, a global resistant problem has been occurring. Some resistances have been shown to be the direct consequence of the widespread and indiscriminate use of antimicrobial agents which allows pathogens to develop the ability to circumvent these administered drugs.[2-4] The Centers for Disease Control and Prevention (CDC) reported that in 2019 more than 2.8 million antibiotic-resistant infections occurred in the United States, with more than 35,000 patients dying as a result.[5] One such example of resistance is the Extended-Spectrum Beta-Lactamase (ESBL) enzyme which breaks down the commonly used beta-lactam antimicrobial agents. ESBL producing enterobacteriaceae infections typically require intense intravenous (IV) antibiotic therapy. Sadly, the incidences of ESBL infections have gradually increased since 2012.[5] This in turn leads to higher costs for treatment, prolonged hospitalization, and increased mortality risk.[6,7] There is an urgent need to ameliorate antimicrobial agents to assist in the fight against these pathogens.

From a global perspective the World Health Organization (WHO) developed the Global Antimicrobial Resistance Surveillance System (GLASS) and Global Action Plan to tackle this resistant crisis. GLASS promotes a standardized approach to collect, analyze, and share resistance data at a global level. It aims to detect emerging resistance and to prevent an international spread. The Global Action Plan is designed to improve awareness and understanding of antimicrobial resistance, reduce infection incidence, optimize the use of antimicrobial agents, and develop the economic case for sustained investment in medications for all countries in need.[7-9]

Alternatively, from a scientific perspective the development of the next generation of antimicrobial agents are through diverse approaches such as the AMR Action Fund. This fund is a partnership of large pharmaceutical companies such as Novartis, Merck, Pfizer, Teva, Taketa, etc., with the goal to bring two to four new antibiotics to market by 2030. This strategy enables different groups to collaborate and hopefully accelerate the research and development of novel antimicrobials.[10]

Munita and Cesar detailed the evading mechanisms of pathogens as either chemical alteration, destruction of the antimicrobial molecules, and/or decreased drug penetration.[11] From understanding the eluding mechanism of pathogens, the redesign or modification of various antimicrobial agents is underway. Some strategies include combining conventional β-lactam antibiotics with enzyme inhibitors (amoxicillin and clavulanate), inhibiting bacterial efflux pumps, and modulating existing cationic antibiotics (CABs) by altering their corresponding counter-anion.[12,13] With the latter approach, it is known that good adsorption of positively charged antibiotics onto the highly negatively charged cell surface of bacteria provide the enhanced antimicrobial efficacy of CABs.[14] There have been numerous reports on the impact of the cationic structure on activity; however, the counter-anion, typically chloride or bromide, also plays a role in the CABs' activity.[15] Indeed, some studies have shown a significant role of the counter-anion on the drug's antibacterial ability. Thus, a simple modification of a counter-anion may lead to a new drug that could combat some resistant pathogens. Herein, is a review comparing inorganic, water-soluble, and lipid-soluble salts of CABs and the role of the anion on their antimicrobial activities.

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2. Inorganic halide counter-anions

Various studies have examined the effects of different halides as counter-anions on antimicrobials. Good adsorption of positively charged antibiotics onto highly negatively charged cell surfaces provides the antimicrobial efficacy of CABs.[14] Thus, the counter-anions associated with these CABs should readily dissociate to allow the positively charged species more opportunity to adsorb onto the cell's surface.[16] Therefore, smaller atomic radii should lead to greater separation from the cationic counterpart, which hypothetically should allow for enhanced antimicrobial activities of the CAB.[16,17] From this perspective, a chloride-containing agent should exhibit more profound antimicrobial activity compared to bromide or iodide. Yoshino *et al*. compared these three halides as counter-anions to evaluate the antimicrobial minimum inhibitory concentration (MIC) of 14 novel-quaternary ammonium silane coupling agents ($[CH_2{=}CHCH_2N^+(CH_3)(C_nH_{2n+1})(CH_2)_3Si(OCH_3)_3]X^-$, n = 10, 12, 14, 16, 18, X = Cl, Br, I).[18] However, when comparing the MIC values of compounds possessing the same alkyl chain length but with different counter-anions, no consistent trend was observed (**Table 1**). The 12-Cl compound had better efficacy against *Staphylococcus aureus* than 12-Br and 12-I. Likewise, 10-Cl and 10-Br showed better activities than 10-I on *S. aureus*. However, unlike the atomic radii assumption, 18-I showed more profound activities against *Micrococcus luteus* than both 18-Cl and 18-Br. Furthermore, 10-Br had enhanced activity against *Bacillus subtilis* than either 10-Cl or 10-I, while 12-Br was most effective compound against *B. subtilis* compared to 12-Cl and 12-I.[18] Thus, other considerations beyond atomic radii need to be evaluated to determine the most effective counter-anions.

Table 1: MIC ($\frac{\mu g}{mL}$) values of 14 novel-quaternary ammonium silane coupling agents ($[CH_2{=}CHCH_2N^+(CH_3)(C_nH_{2n+1})(CH_2)_3Si(OCH_3)_3]X^-$, n = 10, 12, 14, 16, 18, X = Cl, Br, I). Modified from ref 18.

Strain	10-Cl	10-Br	10-I	12-Cl	12-Br	12-I	18-Cl	18-Br	18-I
S.aureus	25	25	100	25	50	50	100	200	200
M.luteus	25	25	100	25	25	50	400	>400	50
B.subtilis	50	25	100	100	25	100	200	400	400

Brunel *et al*. compared chloride and bromide mono-substituted triphenylamine phosphoniums (TPA-P) (**Figure 1**) to investigate the effects of the counter-anions.[19] Ultimately only two compounds could be directly compared. **Compound 1** ($[TPA\text{-}P^+(Hex)_3/Cl^-]$) and Compound 2 ($TPA\text{-}P^+(Hex)_3/Br^-$) contain the same core structure with only the counter-anion modified. These compounds exhibit similar MIC values on all bacterial strains except for *Pseudomonas aeruginosa* where the chloride **Compound 1** showed four times greater activity than the bromide compound **Compound 2** counterpart (**Table 2**). Conversely, **Compound 2** was reported to be more active against *Klebsiella pneumoniae* and *Acinetobacter baumannii*, however the exact magnitude was not determined.[19]

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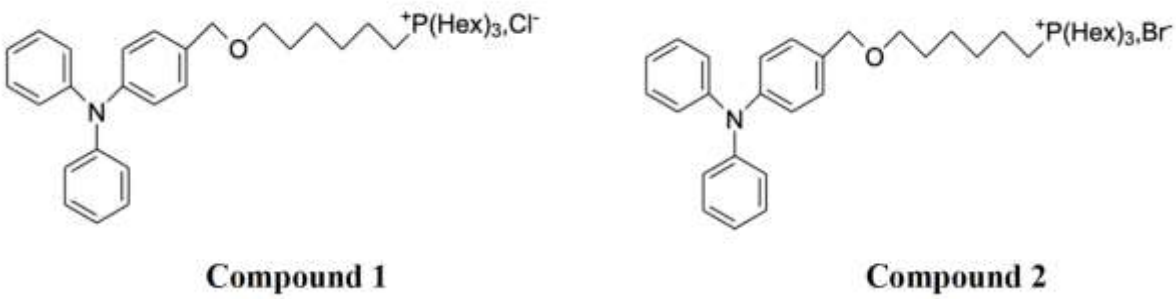

Compound 1 **Compound 2**

Figure 1: Chemical structure of chlorinated (Compound 1) and brominated (Compound 2) TPA-P.[19]

Table 2: MIC values $\left(\frac{mg}{L}\right)$ of TPA-P Compound **1** and **2** against various bacteria. Modified from ref 19.

Bacterial Strain	20,477 *E. faecium*	CIP 7625 *S. aureus*	CIP 82.91 *K. pneumoniae*	ATCC 19,606 *A. baumannii*	CIP 100,720 *P. aeruginosa*	ATCC 13,048 *E. aerogenes*	CIP 54.8 *E. coli*	SA1199 *S. aureus*	SA1199B *S. aureus*
Compound 1	4	2	>64	>64	16	>64	>64	2	2
Compound 2	4	2	64	64	64	>64	>64	2	2

In a different report, the bromide form of a quaternary ammonium polymer (QAP) showed greater antimicrobial effect than its chloride counterpart. Chen *et al.* postulated that the antimicrobial nature of the QAP was depending on the size of the dendrimer, the hydrophobic chain length between the quaternary ammonium groups, and the counter-anion itself.[20] They utilized bioluminescence to monitor for microbial viability.[21] The brominated functionalized propyleneimine (PPI) generation 3 dendrimer with a 14 hydrophobic chain length (D3BrNC14) showed greater efficacy compared to the D3ClNC14 (chlorinated compound). [D3BrNC14 reduced the relative bioluminescence; with a lower value indicating greater inhibition of the bacteria, to about 0.2% in 1 hour while compound with chloride was not effective.] Unlike the previous study, bromide showed to have more efficacy than chloride as a counter-anion.

This phenomenon caught the authors off-guard. They had anticipated that there would be minimal potency difference between these two compounds since both ions have high charge densities, (Relative charge density of Cl^- is 8 and Br^- is 6.[22]) and thus should be similarly dissociated from the QAPs. This hinted that the charge density may not solely explain the antimicrobial efficacy differences.

Kanazawa *et al*. investigated the counter-anions' antimicrobial activity as it relates to the solubility.[16] Here, poly [tributyl(4-vinyl benzyl) phosphonium] salt with various counter-anions (chloride, tetrafluoroborate, perchlorate, and hexafluorophosphate) were evaluated through the log (survivors) versus exposure time against *S. aureus*. They concluded that the chloride showed the most significant antibacterial activity of polymeric phosphonium salts against *S. aureus*. The efficacy on this bacterial strain was ordered, chloride, tetrafluoroborate, perchlorate, then hexafluorophosphate. Kanazawa *et al*. additionally tested the solubility product constant (K_{sp}) of these four compounds in 30°C water to support their solubility hypothesis. The order of K_{sp} value was identical with the antimicrobial activities, suggesting that for this group of CABs, solubility plays a significant role in their efficacy.[16]

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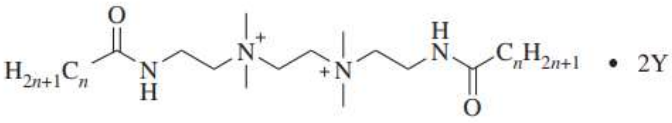
3. Organic counter-anions

Zhou *et al.* postulated that varying organic counter-anions would change the bacteriostatic activities within the same alkyl chain lengths of Gemini quaternary ammonium surfactants.[23] Gemini quaternary ammonium surfactants have versatility and lower critical micelle concentrations than traditional quaternary ammonium monomers. Zhou demonstrated the bacteriostatic counter anion effect against *P. aeruginosa, E. coli, S. aureus*, and *B. subtilis* with formate, acetate, and lactate (**Table 3**). Bacteriostatic effects diminish as the chain length of counter-anions increases. Zhou postulated that this phenomenon was due to a larger diffusion region, which gradually altered the bacteria from obtaining essential nutrients.[23]

Table 3: The bacteriostatic efficiency of Gemini surfactants with different counter-anions at different concentration at 37^0C. Modified from ref 23.



$n = 11, 13, 15 \quad Y = HCOO^-, CH_3COO^-, CH_3CHOHCOO^-$

Structure Gemini surfactants (*n*-2-*n*-2Y)

	P. aeruginosa (%)			*E. coli* (%)			*S. aureus* (%)			*B. subtilis* (%)		
Gemini surfactant	0.01	0.05	0.1	0.01	0.05	0.1	0.01	0.05	0.1	0.01	0.05	0.1
11-2-11-2HCOO$^-$	62.7	78.4	91.6	64.3	86.4	91.3	77.2	80.9	93.2	64.3	86.4	91.3
11-2-11-2CH_3COO^-	60.7	68.5	83.1	60.3	82.8	86.7	75.7	80.0	92.3	60.3	82.8	86.7
11-2-11-2$CH_3CHOHCOO^-$	54.9	67.2	75.3	53.9	74.2	82.7	75.1	79.3	89.3	53.9	74.2	82.7
13-2-13-2HCOO$^-$	63.0	80.2	92.0	66.8	87.3	92.9	78.4	82.4	93.8	66.8	87.3	92.9
13-2-13-2CH_3COO^-	62.5	71.9	87.3	62.7	87.0	90.9	77.4	81.9	93.4	62.7	87.0	90.9
13-2-13-2$CH_3CHOHCOO^-$	58.9	67.0	77.6	54.5	82.0	89.5	76.3	80.8	92.8	54.5	82.0	89.5
15-2-15-2HCOO$^-$	76.0	87.7	92.9	68.8	89.6	93.5	80.2	84.4	94.7	68.8	89.6	93.5
15-2-15-2CH_3COO^-	63.0	84.1	91.2	61.6	88.6	91.9	79.4	84.5	94.7	61.6	88.6	91.9
15-2-15-2$CH_3CHOHCOO^-$	59.1	76.6	81.5	60.9	82.7	90.5	77.9	83.3	94.6	60.9	82.7	90.5
benzalkonium chloride solution	\	\	76.0	\	\	74.0	\	\	79.0	\	\	74.0

4. Inorganic and organic counter-anions

Savic *et al*. complexed the N, N', N'', N'''-tetrakis (2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane (TPMC) with three different AgX salts (**Figure 2**) {X = NO_3^- (**Compound 3**), $CF_3SO_3^-$ (**Compound 4**), and BF_4^- (**Compound 5**)} and compared these different inorganics (**Compounds 3,5**) and organic salts (**Compound 4**).[24] They tested these against five different bacterial strains (*Escherichia coli, P. aeruginosa, A. baumannii, S. aureus*, and *Listeria monocytogenes)* and four different *Candida* species (*C. albicans, C. parapsilosis, C. glabrata,* and *C. krusei*) (**Table 4**).[24]

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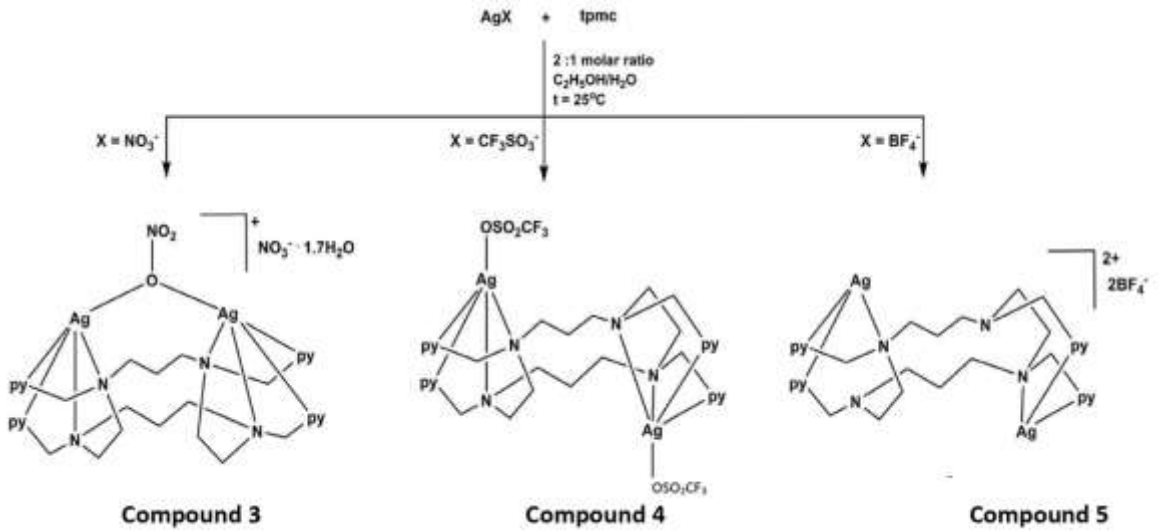

Figure 2: Schematic presentation of the synthetic route and structural formulas of silver(I) complexes 3-5. Modified from ref 24.

Indeed, modifying the counter-anion led to a general improvement in activity compared to free TPMC ligand and silver sulfadiazine (AgSD), with the latter only showing more efficacy over **Compounds 4** and **5** against *C. glabrata*. When comparing the inorganic NO_3^- (**Compound 3**) and BF_4^- (**Compound 5**) salts, variability in potency was depended on the tested organisms. For example, Compound 3 showed better efficacy against *P. aeruginosa, S. aureus, L. monocytogenes, A. baumanni*, and *C. glabrata,* while **Compound 5** showed higher potency against *C. parapsilosis* and MRC-5 cells. The organic counter-anion, $CF_3SO_3^-$ (**Compound 4**) was generally the weakest and only showed superior antimicrobial activities against *C. parapsilosis* over **Compound 3** and against *S. aureus* and *L. monocytogenes* than **Compound 5**. It is arduous to pinpoint the most zenith inorganic counter-anions for CABs, since other factors such as bacterial strains and cationic structures clearly also affect antimicrobial activities.

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Table 4: Antimicrobial activity (MIC) of **Compounds 1-3**, TPMC ligand, and silver(I) sulfadiazine (AgSD) as well antiproliferative effect on the normal human fibroblast cell line (MRC-5). Modified from ref 24.

	Minimal Inhibitory Concentration (MIC, μM)				
Compound	**3**	**4**	**5**	**Tpmc**	**AgSD**
Tested Organisms					
E. coli ATCC 9001	3.2[a]	5.6	3.1	>354	50
P. aeruginosa ATCC 27853	10.7	13	13.1	>354	25
S. aureus ATTCC 259853	26.8	46.7	52.4	>354	75
L. monocytogenes NCTC 11994	26.8	46.7	52.4	>354	NT
A. baumannii ATCC 19606	1.7	5.7	3.2	>354	NT
C. albicans ATCC 10231	2.1	4.6	2.1	354	10
C. albicans isolate 24	2.1	4.6	2.1	177	5.1
C. parapsilosis ATCC 22019	0.9	0.7	0.4	>354	2.5
C. glabrata ATCC 2001	3.3	5.7	13.1	>354	5.1
C. krusei ATCC 6258	0.4[b]	1.5	0.4	>354	2.5
MRC-5 cells	6.4[c]	6.5	6.3	123.9	10

NT-not tested. [a] Standard error is between 1 and 3%. [b] In the case of bolded values, SI > 10 (in respect to MRC-5; SI is selectivity index). [c] Calculated IC_{50} values correspond to concentrations required to inhibit 50% of cell growth.

Kalinowska-Lis *et al*. compared the antimicrobial properties of some inorganic and organic silver-based imidazole salts, [AgX {X = NO_3^- (**Compound** 6), ClO_4^- (**Compound 7**), CF_3COO^- (**Compound 8**), BF_4^- (**Compound 9**), and $CH_3SO_3^-$ (**Compound 10**)}] (**Figure 3A**).[25] They analyzed the MIC (**Figure 3B**) and the minimum bactericidal concentration (MBC) values (**Table 4**) on six microorganisms: *S. aureus, S. epidermidis, E. coli, P. aeruginosa, P. hauseri,* and *C. albicans*.

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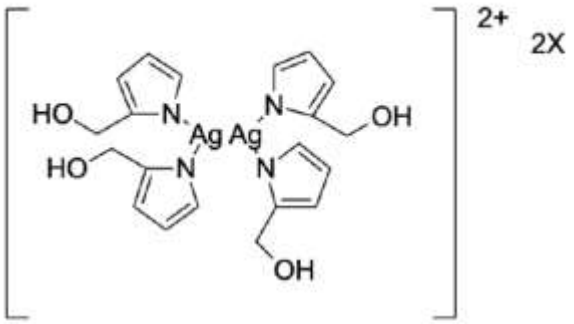
A)



Compound	X
Compound 6	NO_3^-
Compound 7	ClO_4^-
Compound 8	CF_3COO^-
Compound 9	BF_4^-
Compound 10	$CH_3SO_3^-$

B)

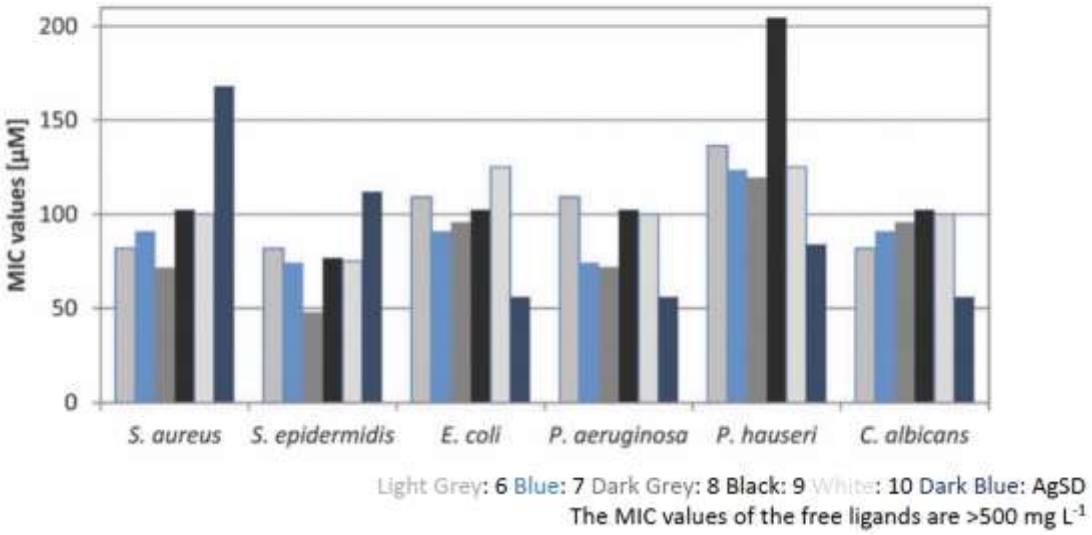


Figure 3: **A**) Chemical structures of inorganic and organic silver-based imidazole salts[24] **B**) Antibacterial activity of silver(I) complexes containing 4(5) -(hydroxymethyl)-imidazole, given as MIC value. Modified from ref 25.

Table 5: MBC (minimum bactericidal concentration) values of the tested compounds against various bacterial strains. Modified from ref 25.

	MBC [μM]					
Tested Compound	*S. aureus* ATCC 6538	*S. epidermidis* ATCC 12228	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 15442	*P. hauseri* ATCC 13315	*C. albicans* ATCC 10231
6	109	109	164	137	219	>273
7	91	124	91	124	173	>248
8	71	96	120	72	144	>240
9	153	150	179	102	230	>256
10	200	153	175	125	175	>250
AgSD	252	224	84	56	84	56

The presence of a noticeable difference in MIC and MBC values amongst the different salts supports the role that the counter-anions effect overall antimicrobial and antifungal efficacy. Here, the organic salt CF_3COO^- (**Compound 8**) was the most active on the gram-positive strains, *S. aureus* and *S. epidermidis*. However, another organic anion, $CH_3SO_3^-$ (**Compound 10**), was the least effective against these strains compared to the

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inorganics: NO_3^- (**Compound 6**), ClO_4^- (**Compound 7**), and BF_4^- (**Compound 9**). Compared to AgSD, altering the counter-anions were deemed ineffective against *E. coli, P. aeruginosa, P. hauseri*, and *C. albicans*.[25] Thus, even though the counter-anions did impact antimicrobial activities they did not constantly increase the potency across the bacterial strains.

Another comparison between inorganic and organic counter-anions supported the previous report.[25] Castiglia *et al.* compared the antimicrobial effect of the counter-anions, chloride and acetate, on the synthetic antimicrobial peptide, SET-M33 (**Figure 4**), a new possible antibacterial candidate for treating multi-drug resistant bacteria.[26] These two salts were tested on *E. coli* and *P. aeruginosa* (**Table 6**).

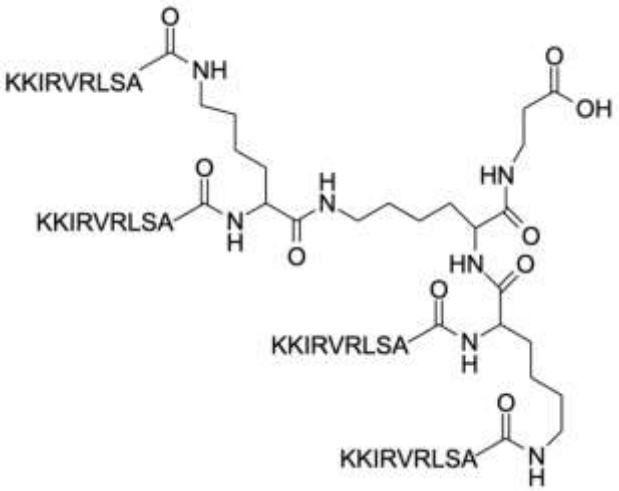

Figure 4: Tetra-branched structure of the antimicrobial peptide SET-M33.[26]

Table 6: MIC values of SET-M33 acetate and SET-M33 chloride. Taken from ref 26.

Bacterial Species	MIC (μM) SET-M33	
	Acetate	Chloride
E. coli TG-1	1.5	1.5
P. aeruginosa PAO-1	3	1.5

Overtly, there was no effect of the counter-anions on *E. coli*, however the inorganic halide salt showed two times greater activity against *P. aeruginosa* than the organic counter-anion, acetate. Similar to the solubility assumption of Kanazawa *et al*.,[16] Castiglia *et al.* postulated that the chloride salt was more active due to it being seven times more soluble than the acetate counterpart.[26] However, the equivalent MIC value against *E. coli* also suggested that solubility was not the only influencing factor to determine the antimicrobial activities of counter-anions.

The inorganic and organic counter-anions on a library of synthetic quaternary ammonium polycarbonates also revealed the variation of the antimicrobial activities. Isik *et al.* compared chloride, citrate, malonate, benzoate, acetate, lactate, trifluoroacetate (TFA), and interestingly penicillin G (**Figure 5**) against *E. coli, S. aureus, P. aeruginosa,* and *C. albicans*.[14] Effects of counter-anions on MIC were not significant against the *S. aureus* and

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C. albicans (**Table 7**), however the Penicillin G showed the most potent effect as a counter-anion on *E. coli* most likely due to its own independent bacterial activity. Conversely, penicillin G was the least effective against *P. aeruginosa*. Additionally, TFA, benzoate, and chloride had enhanced antimicrobial effects as counter-anions to combat *E. coli* only second to penicillin G. However, the most widely used counter-anion chloride was comparably inefficient against *P. aeruginosa*. TFA had an approximately eight times lower MIC value than chloride against this gram-negative bacterial strain.[14]

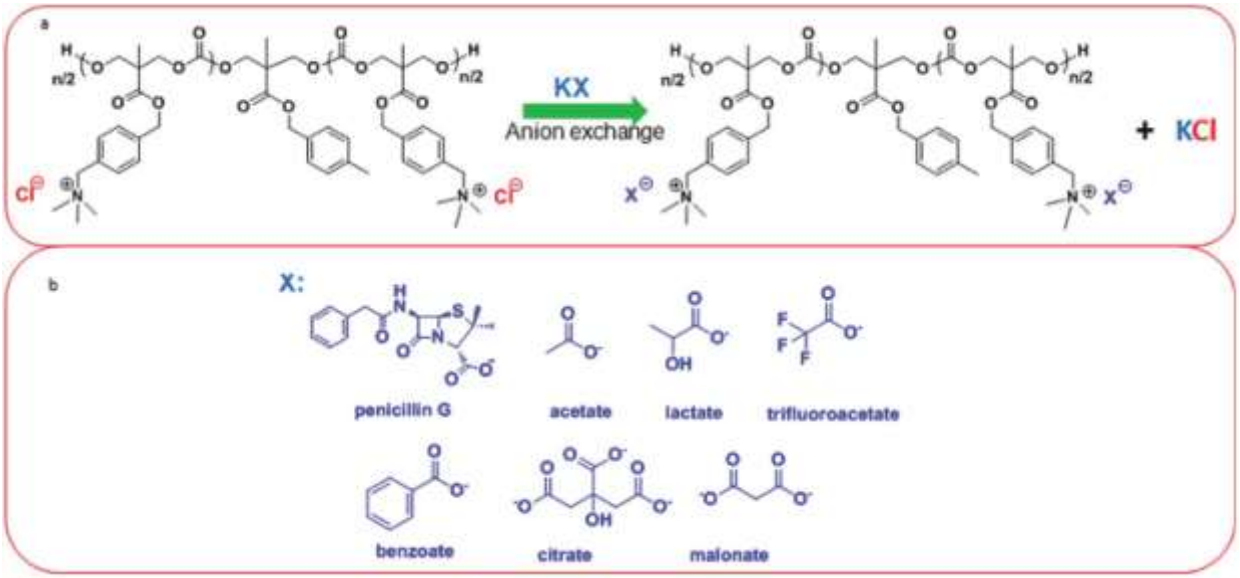

Figure 5: Various anion exchange reactions on quaternary ammonium polycarbonate. Taken from ref 14.

Table 7: MIC values ($\frac{\mu g}{mL}$) of quaternary ammonium polycarbonates with different counter-anions. Taken from ref 14.

Anion	*E. coli* (Gram –)	*S. aureus* (Gram +)	*P. aeruginosa* (Gram –)	*C. albicans* (Fungi)
Chloride	63	16	1000	250
Citrate	125	16	500	250
Malonate	125	16	500	250
Benzoate	63	16	250	250
Acetate	250	16	500	500
Lactate	125	16	250	250
TFA	63	16	125	250
Penicillin G	31	16	>1000	500

Sikora *et al.* investigated the assumption that counter-anions impact the secondary structure of peptides/proteins[27] which it in turn may ultimately affect drug potency.[28,29] Sikora evaluated TFA, acetate, and chloride salts of CAMEL, Citropin 1.1, LL-37, Pexiganan, and Temporin A to compare their anti-staphylococcal activities.[30] The

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antimicrobial peptides showed varying activities with these three anions. Chloride showed consistent superior activity when paired with CAMEL (**Table 8**), however other peptide/anion combinations showed varying effects on different *S. aureus* strains. Concluding which was the most effective counter-anion was not possible through this study. However, Silkora stated that altering the counter-anion is *critical* when testing antimicrobial peptides, suggesting that for each case an independently investigation should be conducted to choose the most effective antimicrobial complex.[30]

Table 8: MIC values ($\frac{\mu g}{mL}$) of tested peptides against various strains. Taken from ref 30.

Peptide and counter-ion	CAMEL			Citropin 1.1			LL-37			Pexiganan			Temporin A		
	TFA^-	AcO^-	Cl^-	TFA^-	AcO^-	Cl^-	TFA^-	AcO^-	Cl^-	TFA^-	AcO^-	Cl^-	TFA^-	AcO^-	Cl^-
S. aureus (29)															
Reference (5)															
ATCC 25923	8	8	2	32	16	16	>	>	>	8	8	8	4	8	4
ATCC 6538	2	≤	≤	16	4	4	128	64	64	4	2	4	4	4	4
ATCC 6538/P	4	1	1	16	8	8	>	>	>	8	4	8	8	4	8
ATCC 9144	8	2	1	32	16	16	>	>	>	8	8	8	8	8	8
ATCC 12598	4	2	1	16	8	8	>	>	>	16	8	8	4	4	4
Clinical (24)															
001N (MRSA)	4	2	2	16	16	16	>	>	>	16	8	8	8	4	8
001S (MRSA)	4	4	2	16	16	16	>	>	>	8	8	8	4	4	4
002N	4	4	≤	16	16	16	>	>	>	8	16	16	4	4	4
002S	4	4	1	16	16	8	>	>	>	16	16	16	4	4	4
004N (MRSA)	4	4	2	16	8	8	>	>	>	8	4	8	4	4	4
005S	≤	≤	≤	2	2	2	2	2	4	1	1	1	4	4	4
013S	≤	≤	≤	2	2	2	2	4	2	1	1	1	4	4	4
015N (MRSA)	4	4	2	16	8	8	>	>	>	16	4	4	8	4	8
017N (MRSA)	4	2	2	8	4	4	>	>	>	4	2	2	8	4	8
017S (MRSA)	2	2	2	4	8	4	>	>	>	4	2	2	8	4	8
024N (MRSA)	≤	≤	≤	2	2	2	2	4	2	1	1	1	4	4	4
030N	4	4	4	16	16	16	>	>	>	16	16	8	4	4	4
033N	4	4	4	16	8	16	>	>	>	16	8	16	4	2	4
039N	4	4	4	16	8	16	>	>	>	8	16	8	4	2	4
043SC (MRSA)	4	4	4	8	8	8	>	>	>	8	4	4	8	4	8
045N	4	4	4	16	16	16	>	>	>	16	8	16	8	4	8
048S	4	4	4	16	16	16	>	>	>	16	16	16	4	4	4
051N (MRSA)	4	2	2	8	8	8	>	>	>	8	8	8	8	4	8
051S	2	2	2	4	4	4	>	>	>	1	1	1	8	4	8
053N	4	4	4	16	16	16	>	>	>	8	8	8	8	4	4
060S	2	1	1	2	1	2	2	2	2	2	1	1	4	2	4
K19N	4	2	1	16	16	16	>	>	>	8	16	16	8	8	8
K46N	4	4	4	16	16	16	>	>	>	8	8	8	8	4	8
K50S	1	1	1	2	1	1	4	2	4	1	2	1	8	4	4
Mean value of MIC	≤ 3.5	≤ 2.8	≤ 2.0	13.0	10.0	10.0	> 411	> 409	> 409	8.4	7.1	7.4	5.9	4.2	5.7

≤ *alone* stands for MIC ≤ 0.25 μg/mL; > *alone* stands for MIC > 256 μg/mL

Salama *et al.* investigated the counter-anions influence on the antimicrobial activities of chitosan guanidinium.[31] Chitosan is a versatile biopolymer allowing for a variety of applications such as modulating adsorption,[32] biomineralization,[33] and drug delivery.[34] Salama suggested that the native chitosan has limited antimicrobial activity, thus modifications with a guanidinium may be advantageous (**Figure 6**). Salama evaluated the antimicrobial efficacy of guanidinium chitosan chloride and acetate salts on *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *C. albicans* (**Figure 7**). The acetate salts (**Compound 11**) showed greater potency and extensive coverage across all bacterial species

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tested compared to its chloride counterpart (**Compound 12**). It has been demonstrated that the acetate ion itself possess inherited antimicrobial activities, which perhaps allowed the improved activity to exist in this study.[35,36]

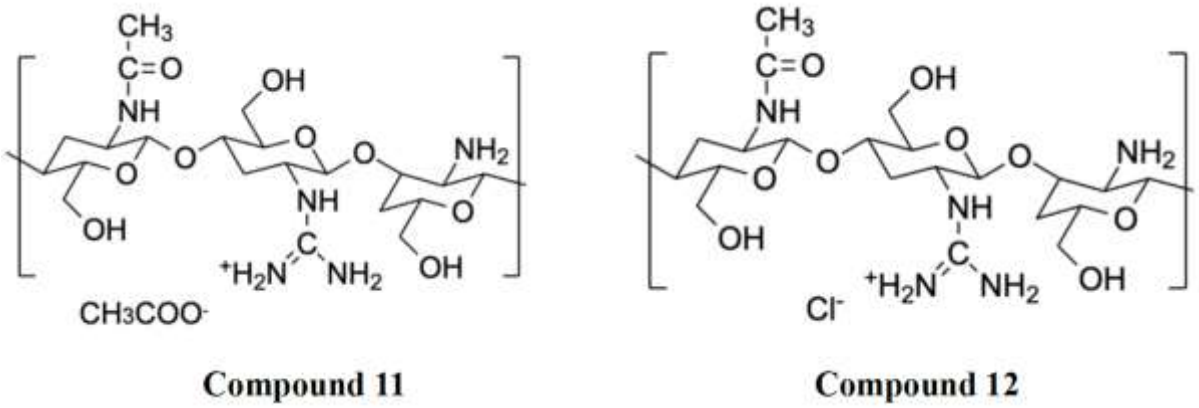

Figure 6: Chemical structure of *N*-guanidinium chitosan derivatives with acetate (Compound 11) and chloride (Compound 12) as counter-anions.[31]

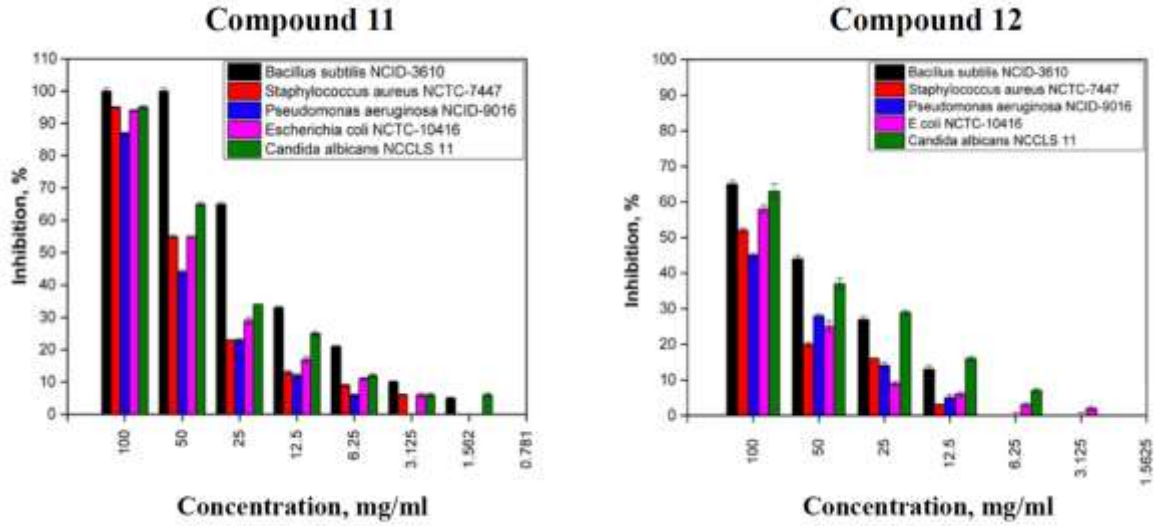

Figure 7: Statistical analysis of MIC values for Compounds 11 and 12 against *E. coli*, *P. aeruginosa, S. aureus, B. subtilis*, and *C. albicans*. Modified from ref 31.

5. Comparing and contrasting inorganic, water soluble, and lipid soluble counter-anions

Kim *et al.* evaluated the antimicrobial activities of styryl-quinolinium-based QACs by exchanging the counter-anions {4-methylbenzene-sulfonate (T), benzenesulfonate (B), 2,4,6-trimethylbenzenesulfonate (TMS), naphthalene-2-sulfonate (N2S), and iodide} (**Figure 8**) and testing against the gram-positive and negative bacteria.[37] Unfortunately, there was no clear trend among the lipid-soluble organic salts {**Compound 13** (T), **Compound 14** (B), **Compound 15** (TMS), **Compound 16** (N2S)} against the gram-positive species (**Table 9**). **Compound 13** (T) was most effective against *S. aureus* ATCC

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29213, *B. cereus,* and *E. facecalis*, whereas **Compound 14** (B) was most effective against *S. aureus* KTCT 1928. **Compound 16** (N2S) was equally effective as **Compound 13** (T) against *S. aureus* ATCC 29213 and *E. faecalis* but had minimal activity against the other two strains. Kim explained that these compounds, with the exception of **Compound 15** (TMS) exerted killing activities against four gram-positive bacteria, suggesting that the molecular weight and size of the counter-anion was not intrinsically related to their activity. No clear "superior" anion was observed against the gram-negative bacteria tested (**Table 10**). Additionally, the iodide salt (**Compound 17**) was only effective against *Salmonella typi* and *S. aureus*: KTCT 1928 and as with previous studies evaluating inorganic salts, no consistent trend was observed.

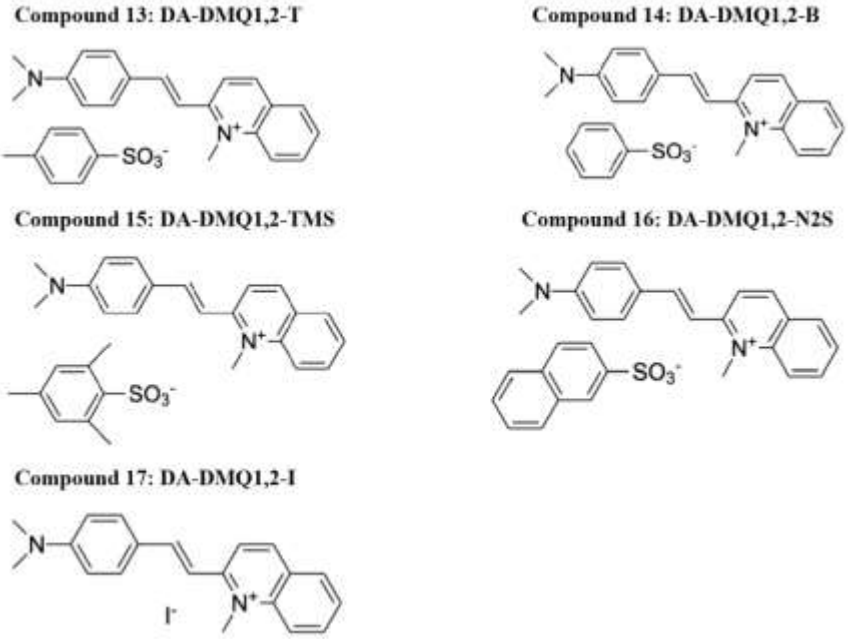

Figure 8: Chemical structures of styryl quinolinium salts with different counter-anions. Modified from ref 37.

Table 9: MIC values ($\frac{\mu g}{mL}$) of quinolinium compounds against gram-positive bacteria. Modified from ref 37.

	S. aureus KTCT 1928	*S. aureus* ATCC 29213	*B. cereus*	*E. faecalis*
Quinolinium				
Compound 13 (DA-DMQ1,2-T)	37.5	4.7	4.7	4.7
Compound 14 (DA-DMQ1,2-B)	4.7	18.75	37.5	4.7
Compound 15 (DA-DMQ1,2-TMS)	150	150	1200	150
Compound 16 (DA-DMQ1,2-N2S)	18.75	4.7	18.75	4.7
Compound 17 (DA-DMQ1,2-I)	2.4	4.7	18.75	4.7

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Table 10: MIC values ($\frac{\mu g}{mL}$) of quinolinium compounds against gram-negative bacteria. Modified from ref 37.

	S. typi	*S.Typhimurium*	*E. coli*	*EHEC*	*P. aeruginosa*	*K. pneumoniae*
Quinolinium						
Compound 13 (DA-DMQ1,2-T)	300	300	18.75	18.75	300	300
Compound 14 (DA-DMQ1,2-B)	37.5	300	75	18.75	150	300
Compound 15 (DA-DMQ1,2-TMS)	>1200	1200	1200	600	600	1200
Compound 16 (DA-DMQ1,2-N2S)	150	600	75	75	1200	1200
Compound 17 (DA-DMQ1,2-I)	37.5	1200	18.75	18.75	300	300

The most extensive comparison study of the role of the counter-anion on antimicrobial activity was conducted by Ingalsbe *et al.*[15] They employed a library of commercially available quaternary ammonium antibacterial agent, tetrabutylammonium (TBA) salt, to investigate the three types of counter-anions.[15] Ultimately 29 TBA compounds were tested against *S. epidermidis* and *E. coli* activity and their activity was determined as a measure of maximum zone inhibition (ZI_{max}) versus the concentration at half the maximum zone of inhibition (K_{ZI}) [($\frac{ZImax}{KZI}$)]. Within the organic lipid-soluble counter-anions tested {trifluoromethane-sulfonate ($CF_3SO_3^-$), perfluorobutane-sulfonate ($C_4F_9SO_3^-$), *p*-toluenesulfonate ($C_7H_7SO_3^-$), benzoate ($C_6H_5CO_2^-$), and triphenyldifluorosilicate ($C_{18}H_{15}F_2Si^-$)}, trifluoromethane-sulfonate showed the greatest antimicrobial activities against *S. epidermidis* (**Table 11**). Again, the different lipid soluble counter-anions showed fluctuating antimicrobial activities. Unlike the Kim study,[37] where the lipid soluble counter-anions of styryl-quinolinium-based QACs showed better antimicrobial activity, here the inorganic salts were superior

Table 11: $\frac{ZI_{MAX}}{K_{ZI}}$ values for library of TBA compounds against both *S. epidermidis* and *E. coli*. Modified from ref 15.

Counter anion		ZI_{max}/K_{zI} (mm µL/µmol)	
Name	Symbol	*S. epidermidis* (ATCC12228)	*E. coli* (K-12(C600))
Iodide	I^-	140.85	0.381
Tribromide	Br_3^-	48.08	37.59
Trifluoromethane-sulfonate	$CF_3SO_3^-$	64.94	0.067
Perfluorobutane-sulfonate	$C_4F_9SO_3^-$	14.62	0
p-Toluenesulfonate	$C_7H_7SO_3^-$	8.55	0
Benzoate	$C_6H_5CO_2^-$	39.81	0.333
Triphenyldifluorosilicate	$C_{18}H_{15}F_2Si^-$	50.76	0

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The inorganic counter-anions were the most effective class of salts (**Table 11**). The TBA-tribromide was substantially the most effective against *E. coli*, while TBA iodide showed the most potency against *S. epidermidis*. Additionally, the water-soluble salt, TBA l-lactate showed better efficacy on *S. epidermidis* than all other organic or inorganics, except iodide. Interestingly, the authors did not expect the high activities of lactate. Sodium lactate does have antimicrobial activity against the gram-positive bacteria but only at higher concentrations.[39] It has postulated that complexation with the TBA allowed the antimicrobial activities to occur at a lower concentration than simple sodium lactate would. The authors noted that there was considerable diversity in activity amongst the counter-anions. TBA cyanide had surprisingly low activity (considering cyanide salts are known cytotoxic agents) than anticipated. Furthermore, hydroxide had only moderate activity against *S. epidermidis* and *E. coli* while in a Sharma *et al*. study of independently complexed poly (4-vinyl 2-hydroxyethyl pyridinium) (PVHEP) with Br^-, OH^-, SH^-, NO_3^-, BF_4^-, and CF_3COO^- investigated against *M. circenelliods, A. niger, and B. coagulans*, the OH^- salt was the most active (**Figure 9**).[40] In that study it was revealed that the PVHEP hydroxide was consistently the most active toward the three strains, however, the other counter-anions showed varying effects depending on the microorganism tested.[40] For example, Cl^- was quite potent against *B. coagulans* but the least effective against *A. niger*.

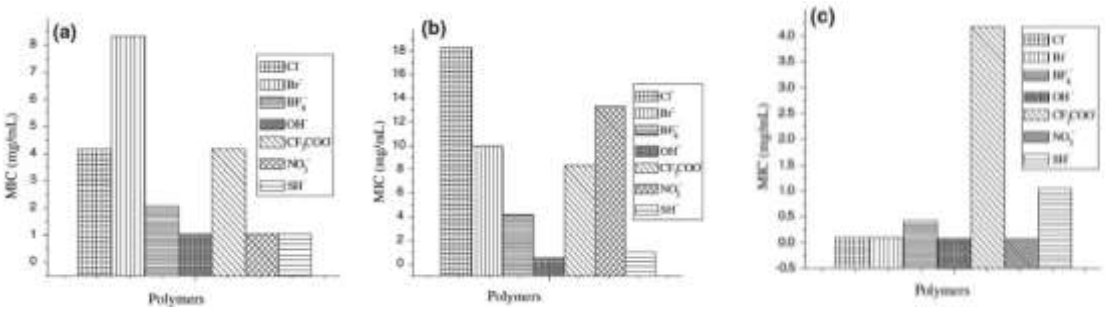

Figure 9: MIC values of PVHEP polymers with different counter-anions against bacterial species: a) *Mucor circenelliods b) Aspergillus nigar c) Bacillus coagulans BTS-3*. Taken from ref 40.

Another report from Garg *et al.* emphasized the role of the OH^- as a counter-anion.[41] Here, Garg replaced Br^- on poly [1-vinyl-3-(2-sulfoethyl imidazolium betaine)] (PSB) with F^-, Cl^-, OH^-, SH^-, SCN^-, NO_3^-, BF_4^-, and CH_3COO^- (**Figure 10**), and tested them against gram-positive, *B. coagulans* (**Figure 11A**) and gram-negative, *P. aeruginosa* (**Figure 11B**). The hydroxide form of PSB was the most potent against *B. coagulans* (**Figure 11A**). The authors suggested that [PSB]OH's potency against *B. coagulans* was due to its high crystallinity and solubility in water leading to more effective interactions with the microbes. However, [PSB]OH was not the most effective salt possessing similar activity against *P. aeruginosa* compared to penicillin, while the [PSB]F, [PSB]NO_3, and [PSB]SH had the greatest activity **(Figure 11B)**. It was postulated that the complexity of the microbe's cell wall led to the variation of the effect, since gram-positive bacteria such as *B. coagulans* contain porous layers which allow for foreign molecules to penetrate easily.[42]

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Figure 10: Chemical structure of PSB with different counter-anions.[41]

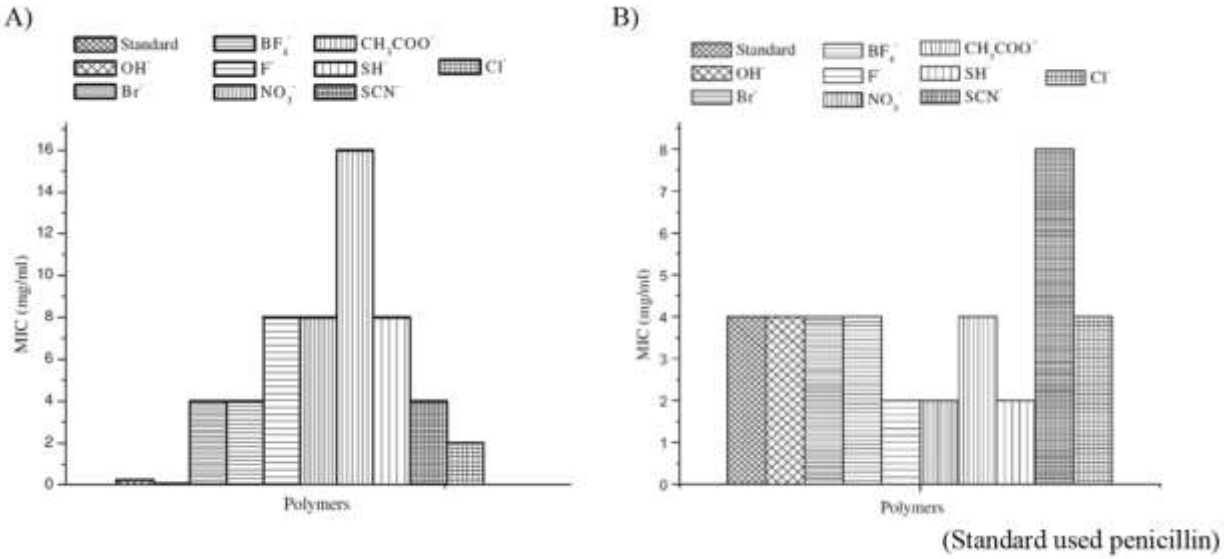

Figure 11: MIC values of the PSBs with different counter-anions against A) *B. coagulans* and B) *P. aeruginosa*. Taken from ref 41.

6. Conclusion

Disdaining the counter-anion when designing antimicrobial drugs may overlook a simple way to increase a drug's efficacy. The influence of the counter-anions, whether increasing or decreasing the antimicrobial activity is crucial to investigate. However, a definitive trend has not been clearly identified. Atomic radii, dissociations, solubilities, and other possible rational all have been suggested, but unfortunately not a single physiochemical parameter has explained the diversity in activity. It seems that performing these onerous and time-consuming studies are necessary in identifying the most active complex. Regardless of this limitation, modifying the counter-anions of an existing drug may strengthen and diversify the available antimicrobial ammunition to fight against resistant pathogens.

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