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Novel Approach of Use of Organic Acids and Salts as a Potent Alternative to Antibiotics

KEYWORDS: Weak acid, Salts, Antibiotics, clinical isolates.

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ABSTRACT *Weak acid preservatives appear to share a common mode of action, despite their various chemical structure. These are increasingly potent as antimicrobial agent at more acidic pH value and appears to share a common mode of action. The present study was designed to investigate the antimicrobial activity of some weak acids include acetic acid, maleic acid, citric acid, boric acid and 5-sulphosalicylic acid and some salts such as magnesium sulphate, sodium citrate, sodium carbonate, sodium acetate, potassium permanganate against common pathogenic organisms isolated from various clinical samples. Most frequently encountered organisms are E.coli, P.aeruginosa, K.pneumoniae, S. aureus, E. feacalis. The comparitive study between antibiotics with weak acids and salts was done and the results reveal that acetic acid, maleic acid, sodium carbonate respectively and antibiotic Gentamycin and Imipenem was found to be highly effective in controlling the pathogens whereas the Klebsiella shows the greater resistance towards the antibiotic studied.*

Introduction:
The effects of organic acids and their salts as antibacterial agents in reducing the bacterial colonies during storage in food industries, meat industries such as beaf poultry and pork have been largely studied (2). As an alternative to antimicrobial therapy, acetic acid and boric acid have been suggested for local treatment of bacterial infections. Both acids have been shown to exert antibacterial effects on different bacterial species, including staphylococci (16,21). Sodium salts of low molecular weight organic acids; such as acetic, lactic and citric acid have been used to control microbial growth, improve sensory attributes and extend the shelf life of various food system including meat (13,23), poultry (26) and fish (3).

The susceptibility of P. aeruginosa to acetic acid in vitro and use of acetic acid in the treatment of pseudomonal infection of skin and soft tissue has been reported earlier. The documented use of weak acid preservatives to inhibit the growth of microorganisms in food and beverages extends back many centuries. Other weak acid preservatives include acetic acid in pickles, propionic acid in bread and more recently, sorbic and benzoic acid in soft drinks (5).Weak acid preservatives appear to share a common mode of action, despite their various chemical structure. Affected microorganisms are rarely killed but growth is prevented. The mechanism of action of weak acid preservatives is thought to involved diffusion of lipophilic acid molecules through the plasma membrane into the cytoplasm (25). Livestock performance and feed efficiency are closely interrelated with the qualitative and quantitative microbial load of the host animal including the load in the alimentary tract and in the environment (9). Poultry posses a limited natural resistance and immunity against colonization or infection by potentially pathogenic microorganisms (11). Organic acid feed additives have made a tremendous contribution to the profitability in the intensive husbandry and providing people with healthy and nutritious poultry products (19). As a consequence of increasing concern about the potential for antibiotic resistant strains of bacteria, so many of non-therapeutic alternatives, including enzymes, (in)oraganic acids, probiotics, prebiotics, herbs and etheric oil and immunostimulants has been used as feed additives. The impact of acid depends on their chemicals characteristics, thereby controlling invitro and invivo the microbial flora. The key basic principle in the mode of action of organic acid on bacteria is that nondissociated (non-ionised, more lipophilic) organic acids can penetrate the bacterial cell wall and distrupt the normal physiology of certain types of bacteria (8). Organic acid have been used for decades in feed preservation, preventing feed and feed raw material from microbial and fungal deterioration, or improving the shelf life of fermented feed such as silages, liquid feeds and liquid by product. Later, other unique properties of organic acids were also discovered, such as their capacity to inhibit and kill microorganism, even at relatively high ambient pH levels. Also, more insight were gained into the physiological effects of organic acids in the gastrointestinal tract.

Individually hydrogen peroxide (H_2O_2) and sodium bicarbonate ($NaHCO_3$) are known to possess antimicrobial activity against oral microorganisms. (14,18). Carbonic acid salts, such as sodium carbonate and sodium bicarbonate, widely used in the food industry are food additives allowed with no restrictions for many applications under European and North American regulations. (12,16). The antimicrobial activity of these chemicals has been described in vitro and in a wide range of substrates as well. (7).

The multidrug resistant bacteria are emerging worldwide which is a big challenge to healthcare. If we didn't take immediate action then antibiotics may lose their power to cure diseases. (27).

Materials and methods
Sample collection
Samples used for the study were obtained from skin, pus, urine, blood. Sterile swab sticks were aseptically used to collect skin swabs from patients, personnel and fomites. A total of 50 samples were collected. The samples were taken to the laboratory and analyzed further.

Isolation and identification
The swab samples were streaked on MacConkey agar, eosin methylene blue (EMB) agar and mannitol salt agar. The urine sample was streaked on Hi Chrome UTI Agar. The plates were incubated together with the nutrient agar plates used to sample the air at 37°C for 24 hrs. The appearance of the colonies on the selective and on differential media used for isolation. The isolates thus obtained was confirmed on the basis of conventional, cultural and biochemical characteristics referring to Bergy's Manual of Determinative Bacteriology.

Collection and processing of antimicrobial agents
Commercially prepared antibiotics disks (Gentamycin, Eryth-

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romycin, Tetracyclin, Chloramphenicol, Ampicillin, Meropenem, Kanamycin, Cefepime, Nalidixic acid, Ofloloxacin, Methicillin, Imipenem, Vancomycin, Cefotaxime, Norfloxacin) were obtained from pharmaceuticals representatives of the respective drug manufacturers. Weak acid include acetic acid, boric acid, citric acid, maleic acid and 5-sulphosalicylic acid and salts such as magnesium sulphate, sodium citrate, sodium carbonate, sodium acetate, potassium permanganate were further processed for the study. Appropriate dilutions of each selected weak acid were made that is 1%, 2%, 3%, 4%, and 5% in distilled water and were used further for antibacterial activity.

Testing for antibacterial properties

The susceptibility pattern of the test organism to the selected antibiotics and weak acid was tested using the disk paper method for antibiotics (6) and well-in-agar diffusion technique (20) for the weak acid.

Table 1 : Antibiogram pattern of bacterial isolates obtained from various clinical samples

Antibiotics	Zone of inhibition in mm				
	P.aeruginosa	K.pneumoniae	E.coli	S.aureus	E.faecalis
Ampicillin (AMP^{10})	R	R	R	R	R
Cefepime (CPM^{50})	R	R	R	12	6
Cefotaxime (CTX^{30})	R	R	R	R	R
Chloramphenicol (C^{30})	R	R	16	14	R
Erythromycin (E^{15})	6	8	R	R	6
Gentamycine (GEN^{10})	8	10	14	10	8
Imipenem (IPM^{10})	22	22	18	36	20
Kanamycin (K^{30})	R	R	8	14	10
Meropenem (MRP^{10})	R	R	R	R	R
Methicillin (M^{5})	R	R	R	R	R
Nalidixic acid (NA^{30})	R	R	R	R	R
Norfloxacin (NX^{10})	R	R	R	6	R
Ofloxacin (OF^{2})	R	R	R	10	R
Tetracyclin (TE^{30})	R	R	R	R	R
Vancomycin (VA^{30})	R	R	R	R	R

Where, R – Resistant

Table 2: Zone of growth inhibition (mm) of clinical isolates on different concentration of weak acids.

Weak acid	Dilution	Zone of inhibition in mm				
		P.aeruginosa	K.pneumoniae	E.coli	S.aureus	E.faecalis
Acetic acid	1%	8	R	8	8	6
	2%	10	10	12	12	10
	3%	12	18	14	12	12
	4%	18	18	16	16	16
	5%	26	20	22	20	24
Boric acid	1%	6	R	R	R	R
	2%	10	R	6	6	R
	3%	18	R	8	12	14
	4%	20	12	14	18	18
	5%	22	18	18	22	20
Citric acid	1%	10	11	8	12	11
	2%	16	16	18	14	16
	3%	20	18	20	14	16
	4%	22	18	22	18	18
	5%	26	22	24	24	20
Maleic acid	1%	12	8	12	8	8
	2%	16	10	14	12	10
	3%	18	14	16	18	12
	4%	22	18	20	22	14
	5%	26	20	24	24	20
5-Sulpho salicylic acid	1%	R	10	R	R	8
	2%	R	12	R	8	8
	3%	12	14	12	10	12
	4%	14	18	14	12	18
	5%	20	24	20	26	22

Table 3: Zone of growth inhibition (mm) of clinical isolates on different concentration of Salts.

Salts	Dilution	Zone of inhibition in mm				
		P.aeruginosa	K.pneumoniae	E.coli	S.aureus	E.faecalis
Magnesium sulphate	1%	R	R	R	R	R
	2%	R	R	R	R	R
	3%	R	R	R	R	R
	4%	R	R	R	R	R
	5%	R	R	R	R	R
Sodium citrate	1%	R	R	R	R	R
	2%	R	R	R	R	R
	3%	R	R	R	R	R
	4%	R	R	R	R	R
	5%	R	R	R	R	R
Sodium carbonate	1%	R	R	R	R	R
	2%	R	R	R	R	R
	3%	R	R	R	R	R
	4%	R	R	R	R	R
	5%	12	13	12	14	15
Sodium acetate	1%	R	R	R	R	R
	2%	R	R	R	R	R
	3%	R	R	R	R	R
	4%	R	R	R	R	R
	5%	R	R	R	R	R
Potassium permanganate	1%	R	R	R	R	R
	2%	R	R	R	R	R
	3%	R	R	R	R	R
	4%	R	R	R	R	R
	5%	R	R	R	R	R

Result and Discussion-

The effect of organic acids and their salt as antibacterial agents in reducing the bacterial colonies have been largely studied as an alternative to antimicrobial therapy. Acetic acid and Boric acid have been suggested for local treatment of bacterial infection. During the investigation 50 different clinical samples were analysed for isolation of the organism. The commonly encountered organisms were Escherichia coli, Pseudomonas aeruginosa, Klebsilla pneumoniae , Staphyococcus aureus, Enterococcus faecalis. The investigation was carried out to study the antibacterial study of 4 different weak acids and 15 antibiotics against five pathogenic microorganism. The result was recorded in terms of zone of inhibition. The result revealed that acetic acid and maleic acid at different concentration that is1-5% was found to be highly effective in controlling all the isolates. This results are in accordance with Sloss et al[24].They shows the topical use of acetic acid at concentration between 0.5 -5% eliminates the P. aeruginosa from burn wound infection .At higher concentration of 5% of all the weak acid studied shows equal effectiveness ranging

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the zone size between 20-26mm. Klebsiella does not shows zone of inhibition at lower concentration upto 3% towards the boric acid. P.aeurginosa was found to be highly sensitive to all the weak acids and similar rersult was obtain to Nagoba et al[17] concluding that 3% acetic acid is non-toxic and efficient in controlling pseudomonal infection. P.aeruginosa shows susceptibility towards boric acid and maleic acid at all the concentration used in study and these result approaches with Muhsin et al[15] showed that 2% acetic acid shows antifungal activity.

The test organism shows resistance towards the salts used in study except sodium carbonate at higher concentration i.e. 5% against all organisms. Zainab et al[28] studied most of salts and observed activity against the different organisms.They reported that 5% Na_2Co_3 has antibacterial activity with inhibition zone ranging between 12-15 mm against Klebsiella oxytoca, S. aureus, and Proteus mirablis. Where as compound $K_2Cr_2O_7$ has high activity against and E.coli and P.aeruginosa only. They also reported that Sodium citrate and Sodium acetate has no influence against all isolates. Zainab et al[28]also concluding that sodium acetate has high activity against Serratia liquefaciens with inhibition zone 32 mm but this compound has no effect against P. aeruginosa, Proteus mirablis and S. aureus and also this result may be approach with Sallam[22], showed that sodium citrate can be utilized as safe organic preservatives for fish under refrigerated storage and these salts have antibacterial activity against bacterial types causing spoilage. From the complete study with weak acids and salts it was noticed that weak acids is used as potential alternative to antibiotics but only some salts show antibacterial activity at only higher concentration.

The antibiotics Imipenem and Gentamycin shows significant antibacterial activity in controlling the clinical pathogens where as K.pneumoniae was found to be highly resistant to almost all antibiotics used in study. Followed by K.pneumoniae, E.coli and Enterococci shows resistant towards the antibiotics used. From that it was concluded that the Acetic acid and Maleic acid was effective from 2-5% whereas the two antibiotics Imipenem and Gentamycin shows significant effectivity in controlling bacterial pathogens however the commonly and routinely used antibiotics such as tetracycline, Methicillin, Vancomycine and Cefotaxime was found to be highly resistant and the MRSA resistance strain was studied by Al Masaudi[1], and VER strain was found by CDC[4]. However, these isolates shows resistance towards the commonly used antibiotics Gentamycin, Erythromycin, Tetracyclin, Ampicilin, Meropenem, Cepepime, Nalidixic acid , Ofloloxacin, Methicillin, Impipenen, Cepotaxime, Norfloxacin and the results of antibiotics was in accordance with MRSA studied by Al Masaudi[1] and also approaches to results with VER strain CDC[4].

In conclusion Resistance to antimicrobial agents is a problem in the community as well as in health care facilities. The continuous use of antimicrobial agents increases selection pressure favouring the emergence multiplication and spread of resistant strain. Therefore it is noted that the use of different weak acids and salts is recommended for effective elimination of multidrug resistant strain of the isolates. Further research is needed towards this field.

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