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Susceptibility Profiles of Alum on Bacteria Isolated from Shellfish Bivalve Oyster

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A B S T R A C T

Antibacterial susceptibility profiles of alum were determined against bacteria isolated from shellfish bivalve oysters. In-vitro bioassay using disc and agar well diffusion techniques with different concentrations; 1.5, 2.0, 2.5 and 3.0% of Alum were used to determine the susceptibility profiles of these isolates and compared with standard antibiotic, Ofloxacin (OFL) as control. Diameter of inhibition zones (DIZ) also were determined by measuring with a meter rule. Alum exhibited high levels of sensitivity on *Proteus* sp (18.0mm), *Bacillus subtilis* and *Staphylococcus aureus* (17.0mm) respectively, *Escherichia coli* and *Klebsiella* species (16.0mm) and *Pseudomonas aeruginosa* and *Vibrio* species (13.0mm) respectively. Its inhibitory potency on Gram negative and Gram positive bacteria suggests broad spectrum activity and can be used as a novel and emerging antimicrobial agent in food systems to combat the effects of some spoilage or biodeteriorative bacteria and foodborne pathogens. Ofloxacin's highest sensitivity profiles to test bacterial isolates suggest broad spectrum antibiotic activity and underscore the fact it could be proficient as the drug of choice for management of patients following the consumption or outbreak of foodborne illness due to these bacterial pathogens.

Introduction

Alum is a salt which is a combination of an alkali metal such as sodium, potassium or ammonium and a trivalent metal; aluminum, iron or chromium. This compound conforms to the general formula $KAl(SO_4)_2$ and also known as aluminum potassium sulphate. Alums are white crystalline anhydrous double salts which contain two different cations with the general formula; $M^+ M^{3+} (SO_4)_2 . 12H_2O$. Depending on the amount of water molecules present, these hydrates are represented by the chemical formulae $KAl(SO_4)_2.12H_2O$ or $K_2SO_4.Al_2(SO_4)_3 . 24H_2O$ (Chang, 2005).

The powder form, made up of crystals has a melting point of 92.5°C (198.5°F) and can readily dissolve in water. This compound is hygroscopic and has a property known as astringency, which is an ability to constrict body tissues and restrict the flow of blood. Alum has been recommended by the U.S Food and Drug Administration (FDA) as a category 1 active ingredient in mouthwashes (Olmez *et al*., 1998), used for the treatment of burns, ulcers in the oral cavity with anticariogenic effect (Mourughan and Suryakanth, 2004) for treatment of haemorrhagic cyctitis and paediatric cough

(Nina, 1997; Bestoon, 2012) and traditionally used for removal of slime on snails in Nigeria. Its synergistic effects with different leaf extracts of guava (*Psidium guajava*) on microorganisms has been reported also (Amadi *et al*., 2016) and used topically to perform deodorant, antibacterial and astringent functions. It is bacteriostatic and reportedly acts on cell surfaces and interstitial spaces with very low permeability into cells and little chance of systemic absorption (Levine, 1985; Alzomor *et al*., 2014).

Currently, sourcing for antimicrobials that will prevent biodeterioration of oysters without adversely affecting the organoleptic properties has become of paramount importance. Potassium aluminum sulphate (Potash alum or Alum) has been used for the improvement and preservation of foods as well as in extension of shelf-life of oysters (Ihediohanma, 2009; Nwosu, 2010; Efiuvwevwere and Amadi, 2015), cosmetics (Alzormor *et al.*, 2014), domestic and industrial water treatments (Potter and Hotchkiss, 2007; Tai and Baqai, 2007). However, based on earlier reports that it has some potential inhibitory capabilities on microorganisms (Dutta *et al*., 1996; Ahmed, 2011) the present investigation is to determine its efficacy on microbes isolated from seafood, e.g., oysters. Presently, there is paucity or no information on the susceptibility profiles of Alum on bacteria isolated from seafood particularly mangrove oysters. Therefore, the objectives of this study were to investigate the antibacterial activity of different concentrations of Alum on microorganisms isolated from postharvest molluscan bivalve, oyster (*Crassostrea gasar*).

Materials and Methods

Preparation of test microorganisms

Subculture of pure colonies of each bacterial isolate obtained from processed mangrove oysters were prepared on Mueller Hinton agar (MHA, Titan Biotech Ltd. Bhiwadi-301019, Rajasthan, India.) and incubated overnight at 37°C. Each growth medium of 10.0mL physiological saline with 10µL bacterial suspension was adjusted to 0.5 McFarland turbidity standards prior to inoculation (Ochei and Kolhatkar, 2008).

Preparation of potassium aluminum sulphate (Alum) and Paper disc

Potassium aluminum sulphate (Vickers Laboratories, Ltd, England.) were made into solution by dissolving 1.5g in 100mL of sterile distilled water to obtain a concentration of 1.5% and repeated to obtain 2.0, 2.5, 3.0% (w/v) respectively. The paper discs were made from Whatman No. 1 absorbent filter paper as described by Ochei and Kolhatkar (2008). The paper discs were dispensed in batches of 40 in Petri-dishes and sterilized at 160°C for 1hour. A 0.2mL of each of the concentration was added into the plate of 40 discs, each containing 0.005mL (5µL) of the Alum concentration (Taiwo *et al.*, 2007). These were stored in wet condition in sterile plates in the refrigerator 4°C until usage.

Susceptibility testing procedure for the Alum concentrations and Ofloxacin

The antimicrobial susceptibility test was performed by the disc and agar well diffusion methods (Bauer *et al.*, 1966; NCCLS, 1999; CLSI, 2011). About 10µL of each of the bacterial suspensions from the overnight culture were spread-plated on Mueller Hinton agar (MHA) using sterile glass spreader (Selvamohan *et al*., 2012) and allowed to dry for 2 to 5 minutes. Paper discs impregnated with Alum concentrations and a disc of commercially supplied Ofloxacin (OFL (5µg, control) (Abtek Biologicals Ltd., Uk) were placed on the surface of MHA with sterile

forceps. Whereas for agar well diffusion method, 0.1mL of various Alum concentrations were dispensed into four wells of 6mm diameter (made using sterile cork borer) equidistant of from each other respectively. Duplicate plates were incubated at 37℃ for 24hours. The diameter of inhibition zones (DIZ) were measured with a transparent ruler and expressed in millimeters (mm). The mean and standard deviation values of DIZ were calculated and compared with Ofloxacin. Interpretation of results was based on the zones of inhibition, susceptible or resistant (Smith, 2004; Cheesbrough, 2006; Forbes *et al*., 2007).

Statistical analysis

All data were obtained from at least two replicated experiments and the mean and standard deviation values estimated using SPSS.

Results and Discussion

Susceptibility patterns of the bacterial isolates were influenced by concentrations of Alum, the higher the concentration the higher the inhibitory action irrespective of the type of isolate (Table 1). *Proteus* sp (18mm) and *E. coli* (15mm) and *Bacillus subtilis* (14mm) showed highest sensitivity at Alum concentration of 3.0% whereas inhibitory activity was least on *Klebsiella* species. At 1.5-2.5% Alum concentrations almost all the bacterial isolates showed marginal differences with increased antibacterial activity. Ofloxacin exhibited the highest sensitivity against *Klebsiella* sp (31mm) and *Escherichia coli* (30mm) respectively with least activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa* (16mm) respectively (Table 1).

Using the agar well diffusion method (Table 2), a similar trend was observed but with much higher zones of inhibition particularly at 3.0% alum concentration with *Proteus* species showing the highest mean DIZ value of (18mm), *Staphylococcus aureus* (17mm), *E. coli* and *Klebsiella* species (16mm) as well as *Pseudomonas aeruginosa* and *Vibrio* species (13mm) respectively. This result demonstrates enhanced inhibitory or antibacterial activity of Alum by agar well diffusion method.

The incidence of large DIZ against *Proteus, E. coli, Bacillus subtilis, Staphylococcus aureus*, etc, demonstrates the antibacterial potential of alum as a new or alternative antimicrobial agent against the emerging multi-drug resistant microorganism in food ecosystems. These opportunistic bacteria and/or pathogens have also been reported to pose serious threat to environment and human health (Cabral, 2010; Chen *et al*., 2013; Ghaderpour *et al.*, 2014; Amadi, 2016). With regard to safety concerns, alum concentrations above 3.0% have been considered non-cytotoxic and antitumorigenic in animals/humans (Oneda *et al.*, 1994).

The susceptibility of *Staphylococcus aureus, E. coli, Klebsiella, Pseudomonas aeruginosa* and others to alum treatment in this study agrees with those previously reported (Bestoon, 2012; Bnyan *et al*., 2014) which presupposes that dipping or pickling in alum enhanced microbial safety of oysters prior to consumption (Efiuvwevwere and Amadi, 2015). Furthermore, this inhibitory ability suggests that it can be used to ameliorate and combat biodeteriorative changes or spoilage of raw and processed oysters as well as several oyster–associated foodborne pathogens.

The sensitivity of alum against Gram negative and positive bacteria (though more efficacious on Gram negative) indicates broad spectrum antibacterial activity which corroborates earlier findings (Dutta *et al.*, 1996; Ahmed,

2011; Bnyan *et al.*, 2014) and further validates its use as a preservative agent (Efiuvwevwere and Amadi, 2015). The high level susceptibility profiles of Ofloxacin exhibited by large DIZ values against all the test bacterial isolates support the fact that tested standard antibiotics should be the most preferred drug of choice for oyster-associated foodborne illnesses.

Table.1 Antibacterial activity of Alum on bacterial isolates from oysters

*Alum / Bacteria	Diameter of inhibition zone (DIZ) in mm (Mean SD)				
	1.5	2.0	2.5	3.0	OFL (5µg)
Proteus sp	8±0.5	9±1.0	11±0.6	18±0.0	26±0.0
E. coli	8±0.7	9±0.9	11±0.5	15±1.4	30±0.0
B. subtilis	8±0.4	9±1.2	11±1.2	14±0.5	26±0.7
Klebsiella sp	8±0.2	9±0.5	11±1.0	10±1.0	31±0.0
S. aureus	8±0.5	9±0.7	10±0.7	12±1.3	16±1.4
Vibrio sp	8±0.7	9±0.2	10±0.7	11±1.0	23±1.4
P. aeruginosa	9±1.2	10±1.5	11±1.8	12±1.0	16±0.7

Legend: [1] = Disc diffusion method; *Alum = Potassium aluminum sulphate concentration (%); OFL = Ofloxacin (control); SD = Standard deviation

Table.2 Antibacterial activity of Alum on bacterial isolates from oysters

*Alum / Bacteria	Diameter of inhibition zone (DIZ) in mm (Mean SD)			
	1.5	2.0	2.5	3.0
Proteus sp	8±2.5	9±1.0	11±0.8	18±1.7
E. coli	8±0.5	11±2.2	11±1.0	16±1.4
Bacillus subtilis	11±0.9	13±2.9	15±1.0	17±1.7
Klebsiella sp	11±2.5	12±2.1	14±2.4	16±3.3
S. aureus	8±1.4	11±1.0	12±0.8	17±4.5
Vibrio sp	9±0.6	10±1.0	12±1.3	13±1.0
P. aeruginosa	9±0.5	11±0.8	12±0.6	13±1.9

Legend: [2] = Agar well diffusion method; *Alum = Potassium aluminum sulphate concentration (%); SD = Standard deviation

In conclusion, potassium aluminium sulphate (Alum) exhibited broad spectrum antibacterial potency against test bacteria but much more on the Gram negative than Gram positive using in-vitro susceptibility tests but more efficacious with agar well diffusion method. Thus, the present study demonstrates that alum can be used as a novel and emerging antimicrobial agent in food systems to combat some spoilage bacteria and foodborne pathogens. The highest level of antibacterial profiles shown by Ofloxacin (OFL) against all the test bacterial isolates suggest broad spectrum antibiotic activity and should be the drug of choice for management of patients following the consumption or outbreak of oyster-associated foodborne illness.

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