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Full Length Research Paper

In vitro evaluation of antibacterial activity of *Parkia biglobosa, Hymenocardia acida* and *Zanthoxylum zanthoxyloides* extracts on pathogenic *Staphylococcus aureus* Isolates

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Abstract
The development of resistance to antibiotics by infectious agents has been a continuous challenge. Thus, this study was designed to evaluate the antibacterial activity of Parkia biglobosa (African Locust Bean tree), Hymenocardia acida (Heart fruit) and Zanthoxylum zanthoxyloides (Candle wood) on pathogenic Staphylococcus aureus. The test organism was isolated and characterized at the Microbiology Laboratory Unit of Federal Teaching Hospital Abakaliki and was re-characterized at the Microbiology Laboratory Unit of Ebonyi State University, Abakaliki using standard microbiology techniques. The leaf and stem bark of the plants were collected from Ameka in Ezza South Local Government Area of Ebonyi State. The herbal plants were sun dried and ground into powder form and extracted with ethanol, methanol, hot and cold water respectively after a period of 24 h. The plant extracts were filtered out using Whatman Number 1 filter paper and allowed to air-dry. The dried extracts were collected and stored in a sterile bottle. The aqueous extracts were tested for antibacterial activity using agar well diffusion, and their zones of inhibition were recorded after 24 h of incubation at 37^0C. From the results obtained, it was observed that only water extracts of the leaf and stem bark of Parkia biglobosa and Hymenocardia acida showed inhibitory activity on pathogenic Staphylococcus aureus whereas their ethanol and methanol extracts showed no activity. The test organism was resistant to all the extracts of Zanthoxylum zanthoxyloides. The minimum inhibitory concentration for the aqueous fraction of the extracts was 12.5 mg/ml. And the multiple antibiotics resistant index of the organism was 0.5. Based on the results of the study, water (hot and cold) extracts of Parkia biglobosa and Hymenocardia acida gave high hope as new agents for the control of pathogenic Staphylococcus aureus. Further studies are required to identify and possibly isolate and concentrate the bioactive components from the plants that were responsible for the antibacterial activity, as they may be potential sources for novel drug development in this era of skyrocketing antibiotic resistance.

Keywords: *Resistance, Herbal plants, Parkia biglobosa, Hymenocardia acida and Zanthoxylum zanthoxyloides*

Introduction

The increase in the level of drug resistant bacteria has negatively affected the efficacy of some available drugs – since these organisms have developed mechanisms that they use to evade the antimicrobial onslaught of these agents. Herbal plants possess several healing powers, and they have been used to meet the primary healthcare needs of man since time immemorial especially in most rural communities. In Nigeria, herbal plants are used in many rural communities to meet the primary healthcare needs of the people – owing to the reported antimicrobial activities of these plants (Ejikeugwu *et al*., 2015; Esimone *et al*., 2007). Medicinal plants have attracted the attention of the medical and pharmaceutical industry due to several evidence-based therapeutic effects of these plants (Savoia, 2012). In general, herbs are plants used to give flavour to food, for their scent or in medicines. Any of the parts of the plants might be considered herbs, which include leaves, roots, seed, root bark, inner bark or other portions (Gibbons, 2004). However, substances obtained from plants have recently generated great interest due to their wide application in treating some infectious diseases (Baris *et al.,* 2006). Plants possess the ability to treat illness (Agbafor *et al.* 2011). The ability could be based on different array of bioactive ingredients that are known to be vital for providing protection against the attack of insects and other organisms (Dixon, 2001; Oseni and Akindahunsi, 2011). Most of these bioactive compounds have been identified and even isolated from various plants that are

known to be medicinal but only few have been exploited for treatment in health care services (Gibbons, 2004). Some medicinal plants that have antibacterial activities include *Hymenocardia acida* (Heart fruit), *Parkia biglobosa* (African Locust Bean tree), and *Zanthoxylum zanthoxyloides* (Candle wood, Senegal Prickly-ash) (Kassim *et al.,* 2005; Millogo-Kone *et al.,* 2006; Ameh *et al.,* 2012). *Parkia biglobosa* is a perennial plant that belongs to *Mimosaceae* family; and it is commonly referred to as African Locust Bean tree. Aqueous fraction of the methanolic extracts of *Parkia biglobosa* stem bark has be reported to exhibit antibacterial activity on organisms like *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* (Millogo-Kone *et al.,* 2006). *Hymenocardia acida* is a dioecious and non-evergreen small tree of (6-10 m) high. It is a very popular African Trado-medicinal plant in Nigeria and other parts of the continents; and the plant are also called "Heart fruit" (Schmelzer, 2008). *Zanthoxylum zanthoxyloides* is native medicinal plant belonging to the family *Rutaceae*. The stem and the root of *Zanthoxylum senegalense* are widely used as local tooth brush in Nigeria precisely by Yoruba nationality and other West African countries like Senegal (Adebiyi *et al.,* 2009; Adegbolagun and Olukemi, 2010). The root and stem bark of the plant provide a warm, pungent and benumbing effect on the palate when chewed; and are mainly used in treating sore gums, toothache and dental caries (Elujoba *et al.,* 2005). The crushed stem bark and root bark are thrown into water to shock fish (Folasade *et al.,* 2006). Herbal plant components including those of *P. biglobosa*, *H. acida* and *Z. zanthoxyloides* considerably have given high potential in the manufacturing of novel products that could be active on resistant pathogenic *Staphylococcus aureus* isolates (Iwu *et al.,* 1999). Thus this study evaluated the effectiveness of *P. biglobosa*, *H. acida* and *Z. zanthoxyloides* on human pathogenic *Staphylococcus aureus*.

Materials and methods

Collection and Processing of Plant Materials: The leaf and stem bark of *P. biglobosa*, *H. acida* and *Z. zanthoxyloides* were collected from Ameka in Ezza South Local Government Area of Ebonyi State, Nigeria. The plant samples were examined and authenticated by a taxonomist, Dr. C.V. Nnamani of Applied Biology Department, Ebonyi State University, Abakaliki, Nigeria.

Test Organisms: The test organisms were pathogenic isolates of *Staphylococcus aureus* obtained from the culture collection section of the Microbiology Laboratory Unit of Federal Teaching Hospital, Abakaliki (FETHA). The test organisms were re-characterized using standard microbiology techniques as was previously described (Onyeagba, 2004; Cheesbrough, 2006).

Preparation of Plant Extracts: The leaf and stem bark of the plants were properly washed with water and cut into pieces; sun-dried and grounded into powdery form using mortar. Four different solvents (hot water, cold water, methanol and ethanol) were used for the extraction. Fifty (50) grams of each ground plant sample was soaked into 250 ml conical flask containing 100 ml each of cold water (28^oC) and hot water (100^oC); and 70 % of methanol and ethanol, and allowed to stand for 24 h. The extract from each solvent was decanted and then filtered with Whatmann No. 1 filter paper. These extracts were concentrated by air-drying to obtain dark, dark-brownish or dark-red pastes. Two (2) grams each of the plant extracts were weighed and introduced into test tubes containing 2 ml of 70 % dimethylsulphoxide (DMSO).

Screening for Antibacterial Activity of Plant Extract: Agar well diffusion method was used to determine the antibacterial activity of the plant extract. The test pathogenic *Staphylococcus aureus* was aseptically streaked using sterile swab sticks on the surface of Mueller-Hinton (MH) agar plates. A 6 mm sterile cork borer was used to bore holes on the agar plate(s), and 4 out of the 5 holes were filled with equal volume of the plant extracts that was dissolved with 70 % DMSO using sterile pipettes (Esimone *et al*., 2010). The 70 % DMSO (which served as negative control) was also poured into one of the wells. The agar plates were kept on a workbench for about 30 minutes for pre-diffusion of the plant extract, and were incubated in upright position at 37^oC for 24 h. The zones of inhibition were measured after incubation using meter rule; and the final inhibition zone diameter (IZD) of each plant extract was evaluated by subtracting the size of the cork borer from the IZD measured (Esimone *et al.,* 2008).

Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extract on the test Organism: The MICs of aqueous fraction of the plant extracts were established by employing tube dilution method, as described by Sahm and Washington (1990). The solution of each extract at varying concentrations: 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.13 mg/ml were prepared. Exactly 1 ml of each aqueous fraction of the extracts was introduced into each test tube which contained 1 ml of nutrient broth; and 1 ml of standardized 24 hour culture of the test organism was also added to it and then mixed thoroughly. The test tubes were incubated at 37^oC for 24 h. A tube containing neither antibiotic nor extract was used as a positive control. The dilution at which there was no detectable growth was considered as the MIC of the extract (Esimone *et al*., 2008; Sahm and Washington, 1990).

Results

The antibacterial activity of *P. biglobosa* leaf extracts on pathogenic *S. aureus* is shown in Table 1. The aqueous (cold and hot water) extracts exhibited activity against the test organism whereas methanol and ethanol extracts had no activity. The hot water extract recorded the highest inhibition zone diameter (IZD) of 23 mm, followed by cold water extract 21 mm while the methanol and ethanol extracts demonstrated no inhibition zone diameter. The positive control (ampicillin) had activity with IZD of 15 mm but the negative control (70% DMSO) showed no inhibition.

Table 1: Antibacterial activity of *P. biglobosa* Leaf extracts on pathogenic *S. aureus*

Extract (10 mg/ml)	Inhibition zone diameter (mm)
Cold water	21
Hot water	23
Methanol	-
Ethanol	-
DMSO	-
AMP	**15**

Key: *— = No inhibition; mm = millimeter; mg/l = milligram per litre; DMSO = Dimethylsuphoxide (negative control); AMP = Ampicillin (positive control).*

The antibacterial activity of *P. biglobosa* stem barks extracts on pathogenic *S. aureus* are shown in Table 2. The aqueous (cold and hot water) extracts exhibited activity against the test organism while methanol and ethanol extracts had no activity. The hot water extract recorded inhibition zone diameter (IZD) of 22 mm, and this was followed by cold water extract which had an IZD of 20 mm. The methanol and ethanol extracts demonstrated no inhibition zone diameter comparable to the positive control (ampicillin) which had activity with IZD of 15 mm.

Table 2: Antibacterial activity of *P. biglobosa* Stem bark Extracts on pathogenic *S. aureus*

Extract (10 mg/ml)	Inhibition zone diameter (mm)
Cold water	20
Hot water	22
Methanol	-
Ethanol	-
DMSO	-
AMP	**15**

Key: *— = No inhibition; mm = millimeter; mg/l = milligram per litre; DMSO = Dimethylsuphoxide (negative control); AMP = Ampicillin (positive control).*

The antibacterial activity of leaf extracts of *H. acida* on pathogenic *S. aureus* are shown in Table 3. The aqueous (cold and hot water) extracts exhibited activity against the test organism whereas methanol and ethanol extracts had no activity. Hot water extract recorded inhibition zone diameter (IZD) of 18 mm, and this was followed by cold water extract which had an IZD of 17 mm. The methanol and ethanol extracts demonstrated no inhibition zone diameter.

Table 3: Antibacterial activity of *H. acida* Leaf extracts on pathogenic *S. aureus*

Extract (10 mg/ml)	Inhibition zone diameter (mm)
Cold water	17
Hot water	18
Methanol	-
Ethanol	-
DMSO	-
AMP	**15**

Key: *— = No inhibition; mm = millimeter; mg/l = milligram per litre; DMSO = Dimethyl suphoxide (negative control); AMP = Ampicillin (positive control).*

The antibacterial activity of *H. acida* stem barks extracts on pathogenic *S. aureus* are shown in Table 4. The aqueous (cold and hot water) extracts exhibited activity against the test organism whereas methanol and ethanol extracts had no activity. Hot water extract recorded inhibition zone diameter (IZD) of 14 mm, and this was followed by cold water extract which had an IZD of 12 mm. The methanol and ethanol extracts demonstrated no inhibition zone diameter. The antibacterial activity of leaf extracts of *Z. zanthoxyloides* on pathogenic *S. aureus* are shown in Table 5. The solvent (cold water, hot water, methanol and ethanol) extracts of the plant exhibited no activity on the test organism. The extracts had no inhibition zone diameter.

Table 4: Antibacterial activity of *H. acida* Stem bark Extracts on pathogenic *S. aureus*

Extract (10 mg/ml)	Inhibition zone diameter (mm)
Cold water	12
Hot water	14
Methanol	-
Ethanol	-
DMSO	-
AMP	**15**

Key: *— = No inhibition; mm = millimeter; mg/l = milligram per litre; DMSO = Dimethylsuphoxide (negative control); AMP = Ampicillin (positive control).*

Table 5: Antibacterial activity of *Z. zanthoxyloides* Leaf Extracts on pathogenic *S. aureus*

Extract (10 mg/ml)	Inhibition zone diameter (mm)
Cold water	-
Hot water	-
Methanol	-
Ethanol	-
DMSO	-
AMP	**15**

Key: *— = No inhibition; mm = millimeter; mg/l = milligram per litre; DMSO = Dimethylsuphoxide (negative control); AMP = Ampicillin (positive control).*

The antibacterial activity of *Z. zanthoxyloides* stem barks extracts on pathogenic *S. aureus* are shown in Table 6. The solvent (cold water, hot water, methanol and ethanol) extracts of the plant exhibited no activity on the test organism.

Table 6: Antibacterial activity of *Z. zanthoxyloides* Stem bark Extracts on pathogenic *S. aureus*

Extract (10 mg/ml)	Inhibition zone diameter (mm)
Cold water	-
Hot water	-
Methanol	-
Ethanol	-
DMSO	-
AMP	**15**

Key: *— = No inhibition; mm = millimeter; mg/l = milligram per litre; DMSO = Dimethylsuphoxide (negative control); AMP = Ampicillin (positive control)*

The minimum inhibitory concentration of aqueous fraction of *P. biglobosa* leaf and stem bark extracts on pathogenic *S. aureus* are shown in Table 7. The minimum inhibitory concentration of cold and hot water extracts was determined at 12.5 µg/ml.

Table 7: Minimum Inhibitory Concentration of aqueous fraction of *P. biglobosa* Leaf and Stem bark Extracts on pathogenic *S. aureus*

Extract	Concentration of Extract (mg/ml)						
	100	50	25	12.5	6.25	3.13	MIC
Cold water	-	-	-	-	+	+	**12.5**
Hot water	-	-	-	-	+	+	**12.5**
Methanol	+	+	+	+	+	+	**ND**
Ethanol	+	+	+	+	+	+	**ND**

Key*: + = Growth; — = No Growth; ND= Not Determined*

The minimum inhibitory concentration of aqueous fraction of *H. acida* leaf and stem bark extracts on pathogenic *S. aureus* are shown in Table 8. The minimum inhibitory concentration of cold water extract was evaluated at 25 µg/ml while that of hot water extract was at 12.5 µg/ml.

Table 8: Minimum Inhibitory Concentration of aqueous fraction of *H. acida* Leaf and Stem bark Extracts pathogenic *S. aureus*

Extract	Concentration of Extract (mg/ml)						
	100	50	25	12.5	6.25	3.13	MIC
Cold water	-	-	-	+	+	+	**12.5**
Hot water	-	-	-	-	+	+	**12.5**
Methanol	+	+	+	+	+	+	**ND**
Ethanol	+	+	+	+	+	+	**ND**

Key: + = Growth; — = No Growth; ND= Not Determined

Discussion

In this study, cold water, hot water, methanol and ethanol extracts of *Parkia biglobosa, Hymenocardia acida* and *Zanthoxylum zanthoxyloides* (leaf and stem bark) were tested on human isolates of pathogenic *Staphylococcus aureus*. The pathogenic isolates of *S. aureus* were collected from Federal Teaching Hospital, Abakaliki (FETHA); and were re-identified using standard microbiological techniques at Microbiology Laboratory of Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. It was observed from this study that the test organism was susceptible to the aqueous (cold and hot water) extracts of the plants; but was resistant to methanol and

ethanol extracts. The highest inhibition zone diameter (IZD) of 23 mm was recorded for hot water leaf extract. The IZD for the hot water stem bark extract, cold water leaf extract and cold water stem bark extracts were 22 mm, 21 mm and 20 mm respectively. The methanol and ethanol extracts of the plants had no inhibitory activity on the pathogenic *S. aureus*. The inhibitory effect of the aqueous plant extracts on the test pathogenic *S. aureus* as obtainable in this present day study concur with the findings of Ndukwe *et al.* (2005). Dey *et al.* (2010) also reported that only organic extracts are used to investigate for the antimicrobial activity of plants due to the poor effect recorded with aqueous extracts. The test organism was susceptible to the aqueous (cold and hot water) extracts; but was resistant to methanol and ethanol extracts. The inhibition zone diameter (IZD) of 18 mm was recorded for hot-water leaf extract, 17 mm for cold-water leaf extract, 14 mm for hot-water stem bark extract and 12 mm for cold-water stem bark extract of *H. acida* plant. But methanol and ethanol (leaf and stem bark) extracts of the plant had no inhibition. However, the result did not agree with the findings by Ogueke *et al.* (2006), which stated that ethanol is the best solvent for extracting most of the plant active substances of medicinal properties. The test pathogenic *S. aureus* was resistant to all the solvent (ethanol, methanol, hot and cold water) extracts of *Zanthoxylum zanthoxyloides* plant. This result is in contrasts with the report by Ajaiyeoba (2002) that aqueous, methanol and ethanol extracts had activity on Gram-positive but not Gram-negative bacteria. The finding also contradicts the report by Rojas *et al.,* (2006), that organic solvent extracts from plants are discovered to show significant antimicrobial activity when compared with aqueous extracts from the same plants. The leaf as well as stem barks of both *P. biglobosa* and *H. acida* aqueous extracts had their minimum inhibitory concentrations on the pathogenic *S. aureus* isolates at 12.5 µg/ml. On the other hand, MIC of methanol and ethanol extracts of this plant could not be determined. Herbal plants including those of *P. biglobosa, H. acida* and *Z. zanthoxyloides* hold sway in revolutionizing medicine especially in this era of antibiotic resistance. It is therefore critical to continue to screen these plants for bioactive agents that could be used to develop novel drugs to contain the effect of global antibiotic resistance.

Conclusion

The result of this preliminary study showed that only water extracts of the leaf and stem bark extracts of *P. biglobosa* and *H. acida* showed inhibitory activity on pathogenic *S. aureus* whereas their ethanol and methanol extracts showed no antimicrobial activity against the test organism. The test pathogenic *S. aureus* was resistant to all the extracts of *Z. zanthoxyloides*; and the MIC of the test plants was recorded at 12.5 mg/ml. The search for novel antimicrobial agents in this era of antibiotic resistance should be channeled to herbal plants with notable antimicrobial activity since some available drugs are becoming less-efficacious for treating some infectious diseases.

Ethics

All the authors read and approved the manuscript and no ethical issues involved.

References

Adebiyi AO, Koekemoer T, Adebiyi AP et al. Antimicrobial and Antioxidant Activities of Crude Extracts of two Nigerian Chewing sticks. *Pharmaceutical Biology.* 2009; 47(4): 320-327.

Adegbolagun OM, Olukemi OO. Effect of light irradiation on the Antimicrobial activity of *Zanthoxylum zanthoxyloides* (lam) Methanolic Extract. *African Journal of Pharmaceutical Pharmacologyi.* 2010; 4:145-150.

Agbafor KN, Akubugwo EI, Ogbashi ME et al. Chemical and Antimicrobial Properties of Leaf Extractcs of *Zapoteca portoricensis. Research Journal of Medicinal Plant*. 2011; 5:605-612.

Ameh SJ, Tarfa FD, Ebeshi BU et al. Traditional herbal management of Sickle Cell Anemia: Lessons from Nigeria. Hindawi Publishing Corporation; Anemia, 2012; 10:1155.

Baris O, Gulluce M, Sahin F et al. Biological activities of the Essential oil and Methanol extract of *Achillea biebersteinii* Afan. (Asteraceae). *Turkey Journal of Biology*. 2006; 30:65-73.

Cheesbrough M. District Laboratory Practice in Tropic Countries, Part 2. low price edition, Cambridge University Press, London, 2006; Pp. 132-157.

Dey SK, Banerjee D, Chattapadhyay S et al. Antimicrobial activities of some Medicinal Plants of West Bengal. *International Journal of Pharmaceutical and Biological Sciences*. 2010; 1:1-10.

Dixon RA. Natural Products and Plant Disease Resistance. *Natural*. 2001; 411: 843-847.

Ejikeugwu C, Umeokoli B, Iroha I et al. Phytochemical and Antibacterial Screening of Crude Extracts from Leaves of Wonderful Kola. *American Journal of Life Sciences*. *Special Issue: Microbiology Research*, 2015; 3(2):5-8.

Esimone CO, Nworu CS, Adikwu MU et al. The effects of a new adoptogen, *Garcinia kola* seed, on the bioavailability of ofloxacin in humans. *Scientific Research and Essays*. 2007; 2(11):482-485.

Esimone CO, Okoye FBC, Odumegwu DC et al. *In-Vitro* Antimicrobial Evaluation of Lozenges containing extracts of Garlic and Ginger. *International Journal of Health Research*. 2010; 3 (2): 105-110.

Esimone CO, Okoye FBC, Nworu CS et al. *In-vitro* interaction between caffeine and some Penicillin antibiotics against *Staphylococcus aureus*. *Tropical Journal of Pharmaceutical Research*. 2008; 7(2):969-974.

Elujoba AA, Odeleye OM, Ogunyem CM et al. Traditional Medicine development for medical and dental primary health care delivery system in Africa. *African Journal of Complementary Medicine.* 2005; 2(1):46-61.

Folasade SI, Olukemi OA, Jones MO. Management of Sickle Cell Anemia in Nigeria with Medicinal Plants: Cationic Evaluation of Extracts and possible effects on the efficacy. *Journal of Biological Sciences*. 2006; 6(1):100-102.

Gibbons S. An Overview of Plant extracts as potential Therapeutics. Expert Opinion, *Therapeutic Pathology*. 2004;13(4):489-497.

Iwu MM, Duncan AR, Okunji CO. New Antimicrobials of Plant origin. In Janick, J. edition, Perspectives on new Crops and new uses.

ASHS Press, Allexandria, V. A., 1999; Pp. 457-462.
Kassim OO, Loyevsky M, Elliott B et al. Effects of Root extracts of *Fagara zanthoxyloides* on the *in-Vitro* growths and stage distribution of *Plasmodium falciparum. Antimicrobial Agents Chemotherapy*. 2005; 49(1):264-268.
Millgo-Kone H, Guissou IP, Nacoulma O et al. Study of the Antibacterial activity of the Stem bark and Leaf Extracts of *Parkia biglobosa* (Jacq) Benth on *Staphylococcus aureus. African Journal of Traditional Complement and Alternative Medicine.* 2006; 3(2):74-78.
Nduwe KC, Okeke IN, Lamikanra A et al. Antibacterial activity of aqueous Extracts of selected Chewing sticks. *Journal of Contemporary Dental Practice*. 2005; 3(6):86-94.
Onyagba RA. Laboratory Guide for Microbiology. Revised edition, Crystal Publishers, Okigwe, 2004; Pp. 40-111.
Ogueke CC, Ogbulie J, Njoku HO. Antimicrobial properties and preliminary Phytochemical analysis of Ethanolic Extract of *Alstonia bonnie*. *Nigerian Journal of Microbiology*. 2006; 20:2896-2899.
Osei OA, Akindahunsi AA. Some phytochemical properties and effect of fermentation on the seed of *Jatropha curcas, Latin American Journal of Food Technology*, 2011; 6:158-165.
Rojs JJ, Ochoa VJ, Ocampo SA et al. Screening for Antimicrobial activity of ten Medicinal plants used in Columbian folkloric medicine: A possible alternative in treatment of non-nosocomial infections. BMC *Complement Alternative Medicine*. 2006; 6:1-6.
Savoa D. Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiol*. 2012; 7(8):979-990.
Schmelzer, G. H. (2008). *Hymenocardia acida* tul. In: Schmelzer, G. H. and Gurib-Fakim, A. (Editors). Medicinal Plants/plantes medicinalis l. PROTA, Waganingen, Netherlands, **11** (1): 12-28.
Sahm, D. F. and Washington, J. A. (1990). Antibacterial Susceptibility Test Dilution Methods. In: Manual of Clinical Microbiology. Lennette, E.H., 5th edition, Washington DC. *American Society of Microbiology,* Pp. 1105-1116.