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Epidemiology of Antibiotic Multidrug Resistant genes in Gram Negative Bacteria among Symptomatic Patients with Bacteriuria Attending Federal Medical Center Yenagoa Bayelsa State Nigeria

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Abstract

The global rise in antibiotic resistance among Gram-negative bacteria poses a significant threat to public health, particularly in resource-limited settings. This study investigates the epidemiology of multidrug-resistant (MDR) genes in Gram-negative bacteria isolated from symptomatic patients with bacteriuria at the Federal Medical Center Yenagoa, Bayelsa State, Nigeria. A total of 100 urine specimens were collected from patients within the age range of 13 to 70 years attending Federal Medical Centre Yenagoa. The specimens were analyzed by culture, biochemical tests, and molecular methods. The Antibiotic susceptibility test was done using the Kirby-Bauer disc diffusion method following clinical and laboratory standard institute (CLSI) guidelines. The results showed that 26 bacterial isolates were obtained which include; *Escherichia coli* 14(53.8%), *Klebsiella pneumonia*e 6(23.1%), *Proteus mirabilis* 4 (15.4%), and *Pseudomonas aeruginosa* 2(7.7%). The highest occurring isolate was *E. coli* 14(53.8%) while the lowest was *P. aeruginosa* 2(7.7%). *E. coli* is the predominant isolate in females 9(34.6%). Female had the highest occurring isolates 14(53.5%) compared to male 12(46.1%). The age ranges with the highest isolates was 51-60 years and 61-70 years with 7(26.9%) respectively,

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while the lowest was within the age range of 13-20 years with 1(3.8%). The antibiotic susceptibility tests revealed that *E. coli* showed the highest resistance to ampicillin (85.7%), ceftriaxone (78.57%), piperacillin/tazobactam (64.28%), and cotrimoxazole (64.28%), with notable susceptibility to ciprofloxacin (57.14%) and levofloxacin. *K. pneumoniae* demonstrated strong resistance to ampicillin and piperacillin/tazobactam at 83.33% respectively, while being most susceptible to ciprofloxacin, gentamicin, and azithromycin at 66.7% respectively. *P. mirabilis* exhibited high resistance to ceftriaxone, tetracycline, and azithromycin (75%) respectively, but showed maximum susceptibility to piperacillin/tazobactam, ciprofloxacin, gentamicin, and levofloxacin (75%) respectively. *P. aeruginosa* was most resistant and completely susceptible to multiple antibiotics at 50% and 100%. The resistance genes CTX-M and SHV were present in *P. mirabilis* while TEM was absent. CTX-M, SHV and TEM were not present in *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. The 16S rRNA of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *E. coli, Klebsiella, Pseudomonas,* and *Providencia* sp., and revealed a close relatedness to the *E. coli, K. pneumoniae, P. aeruginosa,* and *Providencia stuartii.* In conclusion, the observed antibiogram profile revealed multidrug resistance among the isolated bacteria and at the same time pathogenic. Therefore, it is recommended to conduct comprehensive surveillance, investigate risk factors, utilize molecular techniques, evaluate antimicrobial stewardship, and promote one health approach to prevent bacteriuria.

Keywords: Gram negative, Bacteriuria, Antibiogram, Resistance genes, Phylogenetic analysis

INTRODUCTION

The search for healing powers in plants is as old as man. People in all continents of the world have long applied poultices and imbibed infusions of hundreds of indigenous plants dating back to prehistorical period. Till date, natural plants of various types are used in traditional African medicine for providing healing to various ailments even before and after the spread of modern and scientific medicine. Attempts have been made to justify, on a scientific basis, the practice of African traditional medicine. Hence some active components of medicinal plants often used which produces certain anti-microbial properties have been identified [1].

Carica papaya belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases [2]. *Carica papaya* is a neutraceutical plant

having a wide range of pharmacological activities. The whole plant has its own medicinal value. *Carica papaya* is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber [2,3]

The black seeds of the *Carica papaya* are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper. Dried *Carica papaya* seeds actually look quite similar to peppercorns and can be used in just the same way. Grinding a couple over a meal, especially protein rich meals, is a simple way to add extra enzymes to your diet and improve your digestive health. The *Carica papaya* seeds are very pungent and peppery, making them almost unpalatable. However the seeds seem to have more potent medicinal values than the flesh. *Carica papaya* seeds have antibacterial properties and are effective against *E. coli, Salmonella* and *Staphylococcus* infections. *Carica papaya* seeds may protect the kidneys from toxin induced kidney failure. *Carica papaya* can eliminate intestinal parasites. Cure for piles and typhoid and anti-helminthic and anti-amoebic properties [2].

The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs. Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths every day. The situation has further been complicated with the rapid development of multidrug resistance by the microorganisms to the antimicrobial agents available which the present study aim to determine the antimicrobial activity of paw-paw (*Carica papaya*) leaves and seed extracts on *Shegilla* and *Salmonella* species.

MATERIALS AND METHODS

Description of the Study Area and Population

Wukari Metropolis is a large town which is the Headquarters of Wukari Local Government Area of Taraba State. The River Donga and River Benue passes through this area [4]. The Local Government Area shares boundary with Benue and Nasarawa state to the south and west respectively. Geographically, Wukari is one of the 16 local Government areas of Taraba state and lies between latitude 7.53' 43'N, longitude 9.47' 59'E [5]. It is one of the major towns in Taraba state and has an area of 4,308km2 and a population of 241,546 in

the 2006 census. The major spoken languages include, Jukun, Hausa, Fulani and Tiv. The predominant occupations of the people are agriculture, commerce and civil service [6,7]. The region's vegetation is similar to that of the Savannah zone, with grass predominating and a few stray tree species [8,4]. The climate has two different seasons: a wet season from April to October and a dry season from November to March during which there is no rain at all. The Jukuns are the dominant ethnic group in the town, which is widely recognized as the administrative center of the Kwararafa Kingdom. Farming, fishing, and animal rearing are the main jobs held by residents of the Wukari local government region [4].

Collection of Plant Materials (*Carica papaya* Leaves and Seed)

The mature leaves and seed of *Carica papya* fruit was collected from Agriculture research farm, Federal University Wukari and air dried, the dried leaves and seed was grinded into fine powder with a mortar and a piston.

Preparation of Leaves Extract

Healthy, fresh young leaves of *Carica Papaya var*. pusa dwarf Linn was collected from Agriculture research farm, Federal University Wukari. The leaves extract was prepared as described by Peter *et al.* [2] with slight modification. The leave was rinsed under tap water and then by double distilled water and dried at room temperature for 15 days. Dried leaves of 20 g were grounded to powder using a mixer grinder. The 20g dried leaves powder was soaked in chloroform for extraction in a rotary shaker for 3-5 days. The extract was then filtered through cheesecloth and the extract was reduced to 10% of the original volume using a water bath at 40˚C finally dried as powder. The same process was used for aqueous extract preparation.

Preparation of Ethanolic and Hot Aqueous of Seed Extract

Preparation of ethanolic and hot aqueous of leaves and seed extract was done similar to the method carried out by Peter *et al.* [2]. The seed extract was prepared with slight modification through hot extraction method. Air dried ripened *Carica papaya* seeds was powdered using mortar and pestle. For aqueous extract, 5g of dried and powdered seeds were mixed in 100 ml distilled water while for solvent extract preparation, methanol was used. The contents were kept in water bath for 1 hour at 40˚C. After cooling both extracts, were filtered successively through ordinary cheese cloth and Whatmann filter paper. The extracts was then air dried using a water bath at 40˚C and dissolved in dimethyl sufoxide (DMSO) to form different concentrations of viz. 25, 50, 75 and 100% aqueous and

ethanolic extracts respectively. The hot water extraction was done at 80°C in a water bath for ½ hours. The extracts were then decanted and filtered through a Whatman filter paper. The filtered extract was then sterilized using a membrane filter and evaporated to dryness at 45°C.

Experimental Organisms

The bacteria isolates employed in this study were gotten from the Department of Microbiology, at University of Wukari, Nigeria from stool samples collected from General Hospital Wukari which is *Shigella* and *Salmonella* species.

Standardization of test organisms

Marcfarland 0.5 turbidity standard was prepared by adding 0.5ml of 1% w/v barium chloride dehydrate ($BaCl_2.2H_2O$) solution and 99.5ml of 1% sulphuric acid (H_2SO_4). This was mixed well and then aliquoted into test tubes identical to the ones used in the preparing innoculum suspensions of the test organisms. A sterile wire loop was used to pick a loopful of inoculums from a pure culture of the test organism it was transferred and suspended in a tube of sterile normal saline (NaCl 8.5g, distilled water 1 litre). The tube was compared with the turbidity standard and the density of the organism was adjusted to that of the standard by adding more bacterial colony or more sterile saline [9].

Determination of antimicrobial activity

Antibacterial activity of the aqueous and ethanolic extracts of the plant sample was evaluated by the agar well diffusion method [10]. Seeded broth culture of 0.2 ml containing 10-7 $cfuml^{-1}$ of the test organism was inoculated in solidified [10] Muller-hinton [7] agar plates. Four or five wells were made in agar layer of each petri dish by a steel borer. To these the aliquots of 100 ml of extract dilutions, reconstituted in 50% ethanol and ethyl acetate organic solvent extracts and distilled water at concentrations of 50, 100 and 200mg/ml was applied in each of the wells in culture plates previously seeded with the test organisms. The cultures were incubated at 37° C of 24 hours. The antibacterial potential of test compound was determined on the basis of diameter of zone of inhibition around the wells [10].

Well Diffusion Assay

An inoculum suspension was swabbed uniformly to solidified Muller-hinton agar for bacteria, and the inoculum was allowed to dry for 5 min., holes of 5 mm in diameter were

made in the seeded agar using cork borer. Aliquot of 20 µl from each plant crude extract was added into each well on the seeded medium and allowed to stand on the bench for 1 hour for proper diffusion and thereafter incubated at 37°C for 24 hour. Respective solvent was used as the negative control and gentamicin (10µg/ml) as the positive control. The resulting inhibition zones were measured in mm [11].

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of extracts was determined using turbidity method in nutrient broth medium. The experiment was conducted according to serial dilution method. The suspension of seeded broth was made by transferring 2 ml of the seeded broth to 100ml of the 0.9% w/v of the sterilized saline solution. The stock solution of test compounds was prepared at concentration of 50 – 200 mg/ml. 0.1 ml normal saline suspension was added to each assay tube.

The procedure was conducted under strict aseptic conditions. The inoculated tubes were kept at 37º C for 24 hours for bacterial assay. After incubation period, tubes were removed and observed for any deposits and shaken to suspend bacteria that might have been settle down. Minimum Inhibitory Concentration (MIC) values were determined by checking for the absence of visual turbidity [10].

RESULTS

Antibacterial activity of ethanolic extract of *Carica papaya* leaves (Table 1): The ethanolic extract was active against *Salmonella spp.* at a concentration of 200 mg/mL, showing a zone of inhibition of 14 mm. The extract showed no activity against *Shigella spp.* at all tested concentrations. The control (gentamicin) was highly active against both isolates.

Table 2 presented the antimicrobial activity of aqueous of *Carica papaya* leaves showing zones of inhibition. The aqueous extract showed no activity against either *Salmonella* spp. or *Shigella* spp. at any concentration. The control exhibited significant activity against both isolates, with zones of inhibition of 24 mm and 48 mm, respectively.

The antibacterial activity of the ethanolic and aqueous extract of *Carica papaya* seed was offered in Table 3 and 4. No activity was observed against both *Salmonella* spp. and *Shigella* spp. at any concentration. The control displayed notable antibacterial activity. The aqueous

extract showed no antibacterial activity against the tested organisms. The control demonstrated significant inhibitory effects.

The Minimum Inhibitory Concentration (MIC) of both ethanol and aqueous bark extract of *Carica papaya* leaves and seed was presented in Table 5 and 6. For the ethanolic extract of leaves, growth was observed at all concentrations for *Shigella* spp., with inhibition at higher concentrations for *Salmonella* spp. The aqueous extract showed varying levels of growth inhibition, with complete inhibition at the highest concentration (200 mg/mL) for both organisms. The ethanolic seed extract showed partial inhibition, with varying growth responses. The aqueous seed extract showed no growth at the lowest concentration for *Salmonella* spp., but growth was observed at higher concentrations.

Table 1: Antibacterial Activity of the Ethanolic Extract of *Carica papaya* Leaves

Ethanolic Extract	Concentration in mg/mL	Zones of inhibition in (mm)	
		Salmonella spp.	*Shigella* spp.
1	50	0	0
2	100	0	0
3	200	14	0
	Control	20	37

Key: Positive control = Antibiotic disc of gentamicin (10µg/ml), mg/mL = milligram per milliliter, mm = millimeter, spp. = species

Table 2: Antibacterial Activity of the Aqueous Extract of *Carica papaya* Leaves

Aqueous Extract	Concentration in mg/mL	Zones of inhibition in (mm)	
		Salmonella spp.	*Shigella* spp.
1	50	0	0
2	100	0	0
3	200	14	0
	Control	24	48

Key: Positive control = Antibiotic disc of gentamicin (10µg/ml), mg/mL = milligram per milliliter, mm = millimeter, spp. = species

Table 3: Antibacterial Activity of the Ethanolic Extract of *Carica papaya* Seed

Ethanolic Extract	Concentration in mg/mL	Zones of inhibition in (mm)	
		Salmonella spp.	*Shigella* spp.
1	50	0	0
2	100	0	0
3	200	0	0
	Control	22	35

Key: Positive control = Antibiotic disc of gentamicin (10µg/ml), mg/mL = milligram per milliliter, mm = millimeter, spp. = species

Table 4: Antibacterial Activity of the Aqueous Extract of *Carica papaya* Seed

Aqueous Extract	Concentration in mg/mL	Zones of inhibition in (mm)	
		Salmonella spp.	*Shigella* spp.
1	50	0	0
2	100	0	0
3	200	0	0
	Control	34	12

Key: Positive control = Antibiotic disc of gentamicin (10µg/ml), mg/mL = milligram per milliliter, mm = millimeter, spp. = species

Table 5: Minimum Inhibitory Concentration of Both Ethanolic and Aqueous Extract of *Carica papaya* Leaves

Extract	Concentration in mg/mL	Organisms	
		Salmonella spp.	*Shigella* spp.
Ethanol	50	-	-
	100	+	-
	200	+	+
Aqueous	50	+	+
	100	+	+
	200	++	++

Key: + = Indicates growth, - = Indicates no growth, mg/mL = milligram per millilitre

Table 6: Minimum Inhibitory Concentration of Both Ethanolic and Aqueous Extract of *Carica papaya* Seed

Extract	Concentration in mg/mL	Organisms	
		Salmonella spp.	*Shigella* spp.
Ethanol	50	+	-
	100	+	+
	200	++	+
Aqueous	50	-	-
	100	+	-
	200	+	+

Key: + = Indicates growth, - = Indicates no growth, mg/mL = milligram per millilitre

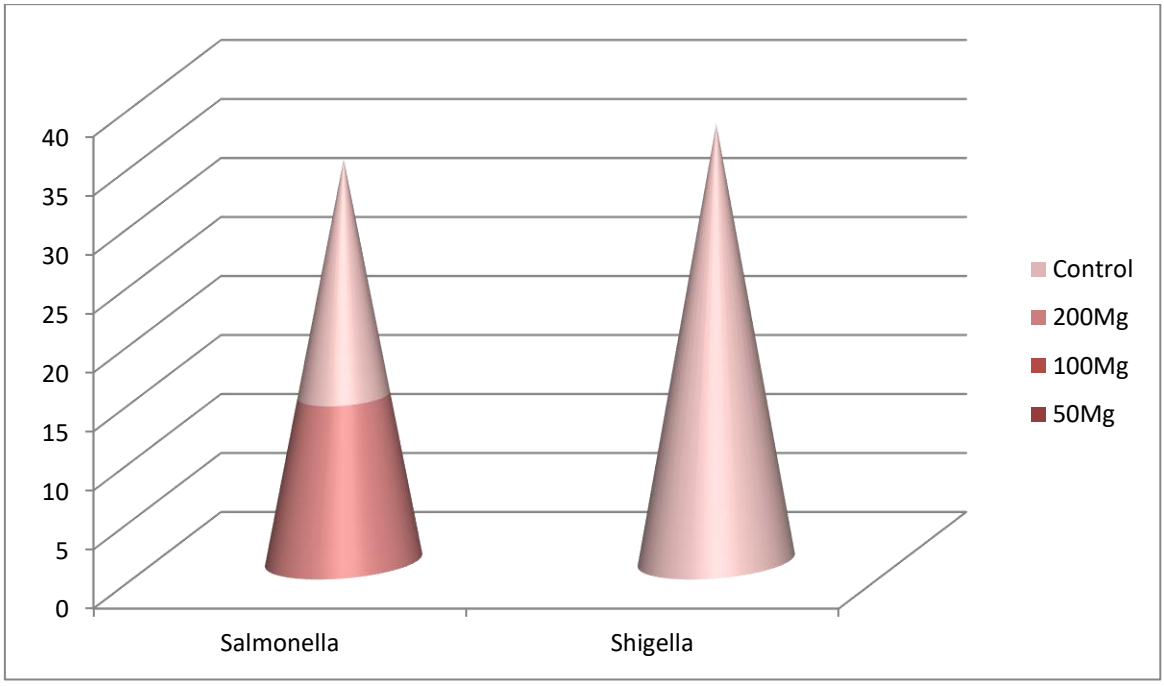

Figure 1: Graphical representation of the antibacterial activity of the ethanolic extract of *Carica papaya* leaves

DISCUSSION

The results obtained from this study reveal that both the leaves and seeds of *Carica papaya* contain bioactive compounds with antimicrobial properties, specifically against *Salmonella* spp. at certain concentrations. The ethanolic extract of the leaves showed notable

antibacterial activity, with a zone of inhibition observed at a concentration of 200 mg/mL. This finding is particularly interesting, as it suggests that the ethanolic extract of *Carica papaya* leaves may contain potent antibacterial compounds effective against *Salmonella* spp., but not *Shigella* spp. This specificity in antibacterial action indicates that the compounds present may target particular bacterial structures or mechanisms unique to *Salmonella* spp.

It is evident from the study that ethanol proved to be a more effective solvent than water for extracting the active constituents of *Carica papaya*. The ethanol extracts displayed higher antibacterial activity compared to the aqueous extracts, which aligns with previous research by Peter et al. [2]. This could be due to the fact that ethanol, being a less polar solvent, may dissolve a wider range of phytochemicals, including those with antimicrobial properties, which are not as readily extracted by water.

The study also highlights the potential therapeutic use of these extracts against *Salmonella* spp., a common cause of enteric fever. The inhibition of *Salmonella* spp. by the ethanolic extract suggests that *Carica papaya* could be explored as a natural therapeutic agent. However, the lack of activity against *Shigella* spp. raises questions about the scope of effectiveness of these extracts against different bacterial pathogens. This differential activity underscores the importance of further pharmacological studies, including the isolation and characterization of specific active compounds. Also further pharmacological evaluations, toxicological studies and possible isolation of the active therapeutic ingredients will be of immense advantage in overcoming the menace of these bacterial diseases. The successful inhibition of these bacteria is a good development, especially when we consider the records of multi-resistance to various conventional antibiotics by bacteria over the years.

The activity indices of the both plant parts with two conventional antibiotics showed that there was intrinsic composition of active ingredients in this plant that could be harnessed in combating the quandary of microbial infections in Africa. The findings support the traditional use of *Carica papaya* for therapeutic purposes, as it has been traditionally employed for its analgesic, antibacterial, and digestive properties, among others [12]. The study's results, particularly regarding the antibacterial activity against *Salmonella* spp., justify the continued exploration of *Carica papaya* in traditional medicine and potential integration into modern medical practices. Anti-cancer activity of better kola has been reported and the use in folklore remedies for the treatment of ailment such as liver disorders, hepatitis, diarrhea, laryngitis, bronchitis and gonorrhea are well known [13].

Despite the promising results, the study also indicates the necessity for further investigation at higher concentrations, especially for the aqueous extracts, which showed limited antibacterial activity. The minimum inhibitory concentration (MIC) data suggest that while there is some inhibition at higher concentrations, particularly against *Salmonella* spp., the activity is not pronounced at lower concentrations. This calls for additional studies to determine the optimal concentration for therapeutic effectiveness and to explore the full range of antibacterial compounds present in *Carica papaya*.

CONCLUSION

The study demonstrated the antimicrobial potential of *Carica papaya* leaves and seeds against *Salmonella* spp. and *Shigella* spp. The ethanolic extracts, in particular, showed promise as antibacterial agents against *Salmonella* spp., supporting their traditional use in treating infections. However, the lack of activity against *Shigella* spp. and the limited effectiveness of aqueous extracts highlight the need for further research, including higher concentration studies and the isolation of active compounds. These findings suggest that *Carica papaya* could be a valuable source of new antibacterial agents, contributing to the development of novel treatments for bacterial infections.

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