Susceptibility of Multi-Antibiotic Resistant Bacteria Strains in Abeokuta, Nigeria to *Aloe vera* Juice

Akinduti Paul Akinniyi[1]*, Ejilude Oluwaseun[1], Deji-Agboola Anotu Mopelola[1], Oladejo Janet Mosunmola[2], Raheem-Ademola Ramota Remi[3], Oluwadun Afolabi [1]

1. Department of Medical Microbiology & Parasitology, 3. Department of Community Medicine and Primary Care, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, P.M.B.2001, Sagamu , Ogun State, Nigeria.
2. Department of Medical Microbiology & Parasitology, University of Ilorin Teaching Hospital, PMB 1459, Ilorin, Kwara State, Nigeria.
***Correspondence**: Akinduti Paul, Department of Medical Microbiology & Parasitology, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria.
e-mail: niyiakinduti@yahoo.com

Abstract

Rapid increasing emergence of antibiotic resistant bacterial strains is a growing problem and a threat to public health both in developed and developing nations of the world. Therefore, the antimicrobial efficacy of *Aloe vera* against multi-resistant bacteria isolates causing various bacteria infections was studied.

The *Aloe vera* juice of 30uL/disc was prepared from a household garden in Abeokuta, Nigeria and commonly used antibiotic discs were tested against bacteria isolates by disc diffusion method. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of used antibiotics were determined. Minimum inhibitory dilution (MID) and minimum bactericidal dilution (MBD) of the *Aloe vera* juice were also determined and interpreted accordingly.

Among the test isolates obtained from different clinical samples, *Eschericial coli* were 56.1%, *K.oxytoca* 19.8% and least proportion of 3.3% was *Citrobacter spp.* All the isolates showed 100% resistant to ceftriazone and nitrofurantoin, while 95.7% and 89.1% to cotrimoxazole and tetracycline respectively by disc diffusion test. All the isolates showed MIC>16ug/ml to ceftriazone, nitrofurantoin and cotrimoxazole, while 62.7% and 69.3% to ciprofloxacin and ofloxacin respectively. All the isolates showed MBC >64ug/ml to ceftriazone, nitrofurantoin, gentamycin and cotrimoxazole while 75.9% and 85.8% to ciprofloxacin and perfloxacin respectively. Of the total test isolates, 76.7% were susceptible to *Aloe vera* juice while 60% showed MID≥6.25% and 46.7% indicate MBD>25%.

Aloe vera extract is effective against multi-resistant enteric bacteria isolates and will serve as a natural remedy for the treatment of prevailing infection caused by these bacteria strains.

Keywords: *Aloe vera* juice, antibiotics, multi-resistant strains, MIC

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Introduction

Rapidly increasing emergence and re-emergence of antibiotic resistant bacterial strains is a growing problem and a threat to public health both in developed and developing nations of the world. It is fast becoming a nightmare to patients, physicians, healthcare managers, and policymakers as it results in poorer health and increased economic loss. This has led to an urgent global call for new antimicrobial drugs, particularly from natural resources mostly plant extracts (1). The advent and continuous use of antibiotics in previous century led to success in limiting most of the prevalent bacterial diseases which affected man and animals in epidemic proportions. At the same time, unregulated use of antibiotics had resulted in emergence of resistance in most bacteria isolates against the commonly used antibiotics and has called for the development of newer antibiotics to check the prevailing infection. The emergence of multi-drug resistant organisms necessitates the search for alternative source of antimicrobial agents (2, 3) mostly from plant extracts.

Plants play a major role in all the traditional system of medicine as it serves as rich source of natural products like vitamins, minerals and other immune-modulators (4). Among the plant considered to be of high benefit to human health and which could cure disease caused by pathogenic microorganisms with little or no side effects is *Aloe vera* (5). It is rich in a wide variety of secondary metabolite such as tannins, terpenoids, alkaloids and flavanoids with excellent anti-microbial properties (6), while its crude preparations are now in use in Medical and Veterinary practice for treatment of various infectious diseases (7). In spite of the increasing bacteria resistance to existing antimicrobial agents, *Aloe vera* is recently being considered as important source in new drug discoveries for treating various ailments related to various bacterial infections with high resistance rate (8).

Therefore, this study is designed to have a more thorough comprehension of antimicrobial efficacy of *Aloe vera* against common multi-resistant bacteria isolates causing various bacteria infections.

Materials and Methods

Plant collection and identification: The *Aloe vera* plant was collected in household garden in Abeokuta. A town located approximately between 77° 30' and 78° 20' longitude and 10° 05' – 10° 09' latitude in South-west, Nigeria. The floristic divisions of Abeokuta consist of dry deciduous thorn forest with annual rainfall of 112mm (10). The *Aloe vera* leave strands were plucked and its juice was aseptically obtained according to Ahmad *et al*., (1998) (9) with minor modifications. Then 30uL of the Aloe vera was pipetted into sterile filter paper disc of 0.5mm diameter.

Collection and identification of isolates: Different bacteria isolates obtained in various clinical samples from major public health facilities in Abeokuta, Nigeria were used as test organisms. Each isolate obtained was cultured on Blood Agar (Oxoid, UK) containing 7% Human blood to test for their haemolytic pattern and MacConkey agar without salt (Oxoid CM 516, UK) for lactose fermentation; and were incubated at 37^{0}C for 18-24hours. Each bacterial isolate was identified according to Cowan and steel (1975) (11).

Antimicrobial susceptibility test: The resistant patterns of the test isolates were tested against commonly used antibiotics such as Tetracycline (30ug), Perfloxacin (5ug), Augmentin (30ug); Ceftriazone (30ug), Nitrofurantoin (200ug), Gentamycin (10ug), Cotrimoxazole (25ug), Ofloxacin (5ug), Amoxycillin (25ug), Ciprofloxacin (10ug) and *Aloe vera* impregnated disc (30uL/disc) using according to Kirby Bauer disc diffusion method on Mueller Hinton agar as described by Fatema *et al*; 2007 (12). Pure bacteria test isolates of 0.5McFarlan turbidity was spread on Mueller-Hinton Agar and antibiotic discs were placed and incubated at 37^{0}C for 18 to 24 hours. The diameters of inhibition zones were interpreted according to CLSI guidelines (13).

Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal Concentration (MBC): MICs of the antibiotic and *Aloe vera* were determined by micro-plate bioassay according to CLSI (2011) (13). Briefly, well 2 to 10 were labelled and into each was pippeted 100µl of sterile peptone water. Then, 100µl of serially diluted antibiotic or *Aloe vera* was put separately into well number 1 to 10, 100µl from well 10 was discarded. Then, 100µl of bacteria isolates adjusted to 0.5MacFarland turbidity was added from wells 1 to 10. Well 11 which serves as positive control contained 100µl of either antimicrobial agent or *Aloe vera* juice and 100µl 0.5Macfarland turbidity bacteria culture. Well 11 which serves as positive control without bacteria growth. Well 12 which served as negative control with bacteria growth contain 100µl of sterile peptone broth and 100µl 0.5Macfarland turbidity bacteria isolates. The culture in the wells was incubated at 37^{0}C overnight. The well having the highest dilution of antibiotic or *Aloe vera* with no bacteria growth were regarded as minimum inhibitory concentration (MIC).

Minimum bactericidal concentration of the antibiotic and *Aloe vera* were determined by plating each MIC broth well into nutrient agar plates and incubated overnight. Lack of growth indicated bactericidal activity of the antimicrobial agent tested while growth

indicated bacteristatic activity (13).The respective MIC and MBC of each isolates was determined and interpreted according to CLSI (2006) recommended guidelines (13).

Statistical analysis: Chi square (X^2) was used to determine the significant of the Aloe vera extract susceptibility against tested resistant isolates.

Result

Table 1 shows, presumed resistant test isolates obtained from different samples sources indicates highest proportion of *Eschericial coli* of 56.1% followed by *K. oxytoca,* 19.8% and least proportion of 3.3% *Citrobacter spp* from different clinical specimens. From Table 2; all the test isolates showed very high resistant pattern to more than three classes of antibiotics. All the thirty isolates showed less than 15mm diameter zone of inhibition to ceftriazone and nitrofurantoin, while 95.7% and 89.1% of the isolates showed same zone to cotrimoxazole and tetracycline respectively. Resistant pattern determined by MIC *>16ug/ml* was observed in 100% isolates to ceftriazone, nitrofurantoin and cotrimoxazole, while least proportion of 62.7% and 69.3% isolates were of MIC*>16ug/ml* to ciprofloxacin and ofloxacin. All the isolates showed MBC *>64ug/ml* to ceftriazone, nitrofurantoin, gentamycin and cotrimoxazole. Of the 30 isolates obtained, 75.9% and 85.8% show MBC *>64ug/ml* to ofloxacin, ciprofloxacin and perfloxacin respectively. The inhibitory activity of *Aloe vera* against resistant bacteria isolates showed in Table 3 indicates 23 out of 30 resistant isolates susceptible to *Aloe vera* juice while 23.3% were resistant. Of the total resistant isolates, 60% show MID$\underline{\geq}$6.25%, while 46.7% showed MBD >25% to *Aloe vera* juice.

Discussion

Increasing bacteria resistant to existing antimicrobial agents is world-wide problem with its attendant prevailing morbidity. It was observed from this present study that there is an increasing rate of antibiotic resistant pattern with 56.1% *Eschericial coli* and 19.85% *Klebsiella oxytoca.* However, the disc diffusion test done against the isolates show high resistant of 100% to ceftraizone and nitrofurantoin while commonly used broad spectrum fluoroquinolone that was believed to be of high efficacy show high resistant rate to 66.0%, 72.6% and 79.2% to ciprofloxacin, ofloxacin and perfloxacin respectively. It was surprising that many of these identified isolates resisted another class of antibiotic; aminoglycoside agents, that is gentamycin with high resistant rate of 89.1%. This suggests an increasing emergence of multi-resistant strains (MRS) of bacteria isolates which is gradually becoming a threat to favourable treatment outcome to common infection in this locality.

Highly diverse antibiotics resistant rates of 100% to ceftraizone, nitrofuratoin and cotrimoxazole with MIC>16ug/ml represent a very critical situation as compared to

investigation from other region of the world reporting resistance to other class of antibiotics. A study by Naik *et al*; (2006) reported high rates of resistant against ampicillin, chloramphenicol and trimethoprim-sulphamethazole (14). This is a fatal challenge for the public health and health institutions due to unguided use of over-the-counter drugs mostly antibiotics. This is an evidence of increasing multi-drug resistant in this locality to many synthetic antimicrobial agents by various bacteria such as enterobacteriacea by production of various resistant enzymes (15).

Despite this prevalence of MRS, the use of plant extracts has shown a great prospect in the treatment of various microbial infections. Herbal drugs are now recently being considered as important sources of new drug for treating ailments related to various bacteria infection (8). *Aloe vera* typically known to have anti-microbial and inflammatory agents is studied for its efficacy against known MRS. Out of the 30 known multi-resistant bacteria isolates obtained, 76.7% were susceptible to *Aloe vera* extract. This results is in conformity to earlier findings of Tian *et al*; (2003) and Agars *et al*; (2005) who found anti-*E.coli* activity and anti-*S.aureus* activity of alcoholic extract of *Aloe vera* extract (16,17). The emergence of these multi-resistant strains with virulence activity had caused increased financial burden due to inactivity of synthetic antibiotics while *Aloe vera* is known to be cost-effective plant agent particularly in developing countries (3).

From this study, it was found that *Aloe vera* exerted strong inhibitory activity against many MRS with 60% showing MID>6.25% while there is significant cidal activity of MBD>25% to 46.7% MRS isolates. This finding is similar to study by Thiruppathi *et al* (2010); who reported antimicrobial activity of the *Aloe vera* juice against *M. smegmatis, K. pnuemoniae, E.fecalis, M. luteus, C. albicans and B. sphericus*, and least inhibitory effect against *M.luteus* (1). This activity could be attributed to a number of pharmacologically active compounds including anthraquinones, aloin, aloe-emodin, aloetic acid, anthracine, aloe mannan, aloeride, antranol, chrysophanic acid, resistanol and saponin (18). Aloin and aloe-emodin possess strong antibacterial and antiviral activities as well as laxative, hepatoprotective and antineoplastic characteristics (19). Aloin and aloe-emodin have been known to have polyphenolic structures, which can inhibit protein synthesis in bacteria cells, thus explaining their antimicrobial activity.

Conclusion

Since *Aloe vera* extract is effective against multi-resistant bacteria isolates, it is a good alternative for the treatment of prevailing infection due to these groups of bacteria. It is cheap to obtain, cost-effective and easily available when compared to synthetic antibiotic and can be useful in a resource limited country.

Table 1: Sample source of the bacteria isolates

	E.coli	*K.*	*Proteus spp*	*P.*	*Citrobacter*	Total
	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
Feces	8(26.7)	1(3.3)	2(6.6)	2(6.6)	1(3.3)	14(46.
HVS	1(3.3)	1(3.3)	0(0.0)	0(0.0)	0(0.0)	2(6.6)
Urine	8(26.7)	3(6.6)	0(0.0)	0(0.0)	0(0.0)	11(36.
Eye swab	0(0.0)	1(3.3)	0(0.0)	0(0.0)	0(0.0)	1(3.3)
Ear swab	0(0.0)	0(0.0)	1(3.3)	1(3.3)	0(0.0)	2(6.6)
Total	17(56.1	6(19.8)	3(9.9)	3(9.9)	1(3.3)	30(100

Keys: N=total number of the isolate; (%) is percentage rate of occurrence.

Table 2: Resistant pattern of the bacteria isolates against commonly used antibiotics

Antibiotics (ug/disc)	***Zone of inhibition (<15mm diameter) n(%)***	***MIC (>16ug/ml) n(%)***	***MBC (>64ug/ml) n(%)***
TET(30)	27(89.1)	26(85.8)	29(95.7)
PFX(5)	24(79.2)	24(79.2)	26(85.8)
AUG(30)	25(82.5)	25(82.5)	28(92.4)
CRO(30)	30(100.0)	30(100.0)	30(100.0)
NIT(200)	30(100.0)	30(100.0)	30(100.0)
GEN(10)	27(89.1)	28(92.4)	30(100.0)
COT(25)	29(95.7)	30(100.0)	30(100.0)
OFX(5)	22(72.6)	21(69.3)	23(75.9)
AMX(25)	27(89.1)	29(95.7)	29(95.7)
CPX(10)	20(66.0)	19(62.7)	23(75.9)

Keys: MIC:minimum inhibitory concentration; MBC: minimum bacteriocidal concentration; TET: Tetracycline; PFX: Perfloxacin; AUG: Augmentin; CRO: Ceftriazone, NIT: Nitrofurantoin; GEN: Gentamycin; COT: Cotrimoxazole; OFX: Ofloxacin; AMX: Amoxycilli; CPX: Ciprofloxacin; n:number of tested bacteria, (%):percentage rate of resistance.

Table 3: Inhibitory activity of *Aloe vera* against resistant bacteria isolates

Isolates	*Aloe vera* inhibitory activity			
	Susceptibility rate n(%)	Resistant rate n(%)	MID≥6.25% n(%)	MBD>25% n(%)
E.coli (n=17)	14(46.7)	3(9.9)	11(36.7)	11(33.3)
Klebsiella spp (n=06)	5(16.7)	1(3.3)	1(3.3)	1(3.3)
Proteus spp (n=03)	1(3.3)	2(3.7)	1(3.3)	1(3.3)
Pseudomonas spp (n=03)	2(6.7)	1(3.3)	4(13.3)	1(3.3)
Citrobacter spp (n=01)	1(3.3)	0(0.0)	1(3.3)	1(3.3)
Total	**23(76.7)**	**7(23.3)**	**18(60.0)**	**14(46.7)**

Keys: n;total number of resistant isolate, %; percentage rate of resistance

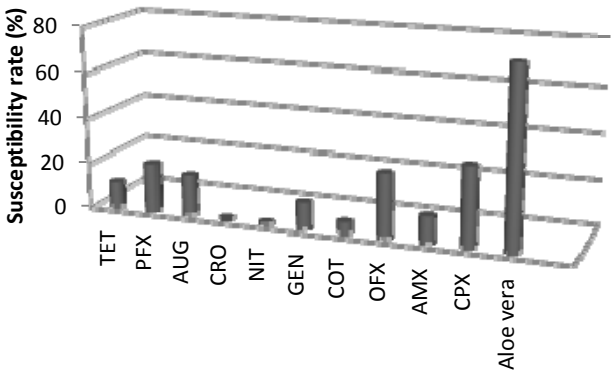

Figure 1. Susceptibility rate of test bacteria isolates to commonly used antibiotics and *Aloe vera* juice.

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