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ANTIBACTERIAL ACTIVITIES OF *Moringa oleifera* (LAM) ON COLIFORMS ISOLATED FROM SOME SURFACE WATERS IN AKURE, NIGERIA

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ABSTRACT

Antibacterial activities of *Moringa oleifera* (LAM) leaf extracts were carried out on coliforms isolated from surface waters using growth inhibition indices based on agar plate technique. Hot water and two organic solvents, namely acetone and ethanol were used for the extraction with 16.67%, 17.86% and 21.62% recovery respectively. The plant extract was also subjected to column and thin layer chromatography for purification and fractionation of the biologically active components. The mean count of coliforms/ml in water samples ranged from 12.80×10^3 cfu/ml to 48.60×10^3 cfu/ml. At 12.5 mg/ml, the active constituents demonstrated higher activity with 11.5 mm zone than crude extracts (7.01 mm, 6.38 mm and 0.0 mm zones in acetone, ethanol and hot water respectively) on Escherichia coli. Gentamycin, the reference antibiotic, had between 2.33 and 24.67 mm inhibitory zones on the isolated organisms. The minimum inhibitory concentration ranged from 4.0 mg/ml to 35.0 mg/ml, while the minimum bactericidal concentration ranged from 5.0 mg/ml to 45.0 mg/ml. The antibacterial activities examined in this study showed that *Moringa oleifera* leaves are capable of exerting inhibitory effect on the target organisms.

Keywords*: Moringa oleifera,* antibacterial, surface waters and coliforms.

INTRODUCTION

Coliform is the name of a test adopted in 1914 by the Public Health Service for the Enterobacteriaceae family. It is the commonly-used bacterial indicator of sanitary quality of water. They are defined as rod-shaped gram-negative non-spore forming bacteria (Willey *et al*. 2008). Coliforms are abundant in the faeces of warm-blooded animals, but can also be found in the aquatic environment, in soil and on vegetation (Willey *et al*. 2008).

Drinking water comes from two major sources: groundwater and surface water (Fetter, 2011). Surface water refers to water occurring in lakes, rivers, streams, or other fresh water sources used for drinking water supplies (Fetter, 2011). Lakes, rivers and streams have important multi – usage components, such as sources of drinking water, irrigation, fishery and energy production (Iscen *et al*. 2008). The impact of these anthropogenic activities has been so extensive that the water bodies have lost their self-purification capacity to a large extent (Sood *et al*. 2008). Contaminated fresh water is a major cause of waterborne diseases and when used in the preparation of food, can be the source of food borne diseases (Willey *et al*. 2008).

Freedom from contamination with faecal matter is the most important parameter of water quality (Scott *et al.* 2003). Human faecal matter is generally considered to be a greater risk to human health as it is more likely to contain human enteric

pathogens (Scott *et al*. 2003). Waterborne diseases contribute to the death of about 4 million children in the developing countries every year and the situation in Nigeria rises steadily (WHO, 2002).

Fortunately, the flora of Africa is rich with a lot of medicinal plants (Sofowora, 1984). There are about 13 species of *Moringa* trees in the family Moringaceae. Of these species, *Moringa oleifera* is the most widely known (Price, 2007). *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics including Nigeria where its leaves are known as "Ewe Igbale" in Yoruba Language. It has an impressive range of medicinal uses with high nutritional value (Farooq *et al*, 2007). *Moringa oleifera* is cultivated and used as a vegetable (leaves, green pods, flowers, roasted seeds), for spice (mainly roots), for cooking and cosmetic oil (seeds) and as a medicinal plant (all plant organs) (Rebecca *et al*, 2006). Studies (Price, 2000; Ajayi, 2008; Bukar *et al.*, 2010; Kasolo *et al.*, 2010) of antimicrobial activities of *Moringa oleifera* have been reported.This study therefore evaluates the antibacterial property of crude and purified extracts of *Moringa oleifera* on some coliforms isolated from surface waters.

MATERIALS AND METHODS

Collection of water samples

Water samples from three surface waters namely Shagari stream, Ala River and Federal University of Technology, Akure (FUTA) stream in Akure, Ondo State, Nigeria were collected in sterile 500 ml sample bottles according to Nurcihan and Basaran (2009) and taken to the Microbiology laboratory of the Federal University of Technology, Akure within 2 hours of collection for microbiological analysis.

Isolation and identification of bacterial isolates

Water samples obtained from surface waters were subjected to microbiological analysis. Ten millilitres of each water sample was diluted in 90 ml of sterile distilled water, followed by fourth to ninth fold serial dilutions (10^{-4} to 10^{-9}). One tenth of a millilitre of the fourth to ninth fold diluents were plated out in triplicates on eosin Methylene blue agar, Salmonella-Shigella agar, Mac Conkey agar and nutrient agar media (Olayemi and Opaleye, 1990). Distinct colonies were subcultured on nutrient agar plates and identified using various morphological and biochemical tests (Fawole *et al*, 2001).

Collection and preparation of plant materials

Fresh leaves of *Moringa oleifera* were collected from a farm settlement at Ado-Ekiti, Ekiti State, Nigeria. The leaves were identified and authenticated at the museum of the Department of Crop, Soil and Pest Management, the Federal University of Technology, Akure (FUTA), Ondo State, Nigeria.

Extracts were prepared as described by Harbone (1998) with slight modifications. The leaves were air dried for three weeks and pulverized using an electric blender (Marlex Electrolyne IS: 250). The solvents used for the extraction were 100% ethanol, acetone and hot water. Exactly 200 g of the powdered leaf was soaked in each solvent. Each solution was allowed to stand for 72 hours, after which it was sieved with a muslin cloth and filtered using No 1 Whatman filter paper. The filtrate was collected in a beaker and concentrated in a *vacuo* using rotary evaporator (Resona, Germany). The extracts were reconstituted in tween 20 (10% v/v) prior to use and sterilized with the aid of membrane filter (0.22 µm). The dry weights of the dried extracts were measured and reported.

Percentage extract recovery = (Dry weight of extract recovered after extraction/ Initial dry weight of plant part) x 100%

Fractionation of extracts

Chloroform fraction (CF) was obtained by fractionating one gramme of the extract using column chromatography (CC) (Si gel column, 60g, 250ml burette). Fractions (100 ml) were eluted using chloroform. Main fractions were pooled together and were re-chromatographed using short column (Si gel column, 30 g, 25 x 1 cm). The second fraction (C2) was further purified using thin layer chromatography (TLC) (Si gel TLC, 60 g, 250 ml burette) using chloroform-methanol (9.5: 0.5 v/v) as the solvent system; it gives a light yellow colour. This was done according to Philip (2003).The procedure was repeated for the

extraction of methanol fraction (MF) where methanol was used as the solvent system. It yielded a grey colouration.

Phytochemical analysis of the plant extract

The extracts were screened for phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroid, alkaloids and glycosides in accordance with Trease and Evans (2004).

Assay for antibacterial activity

Antimicrobial activities of the plant extracts were determined by the Agar Well diffusion method as described by Esimore *et al* (1998). Different concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml of the extracts were used for the bioassay and standard antibacterial agent; gentamycin (10µg) was used as positive control. After incubation, zones of inhibition formed in the medium were measured in millimeter (mm) diameter to determine the antibacterial effectiveness of the extracts.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the leaf extract of *M. oleifera*

The MIC of isolates was carried out using tube dilution technique as described by Doughari *et al* (2007). 0.5 McFarland turbidiometric standard (10^6cfu/ml) was used to standardize the concentration of test organisms (*Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Serratia marcescens, Salmonella typhi, Proteus vulgaris, Shigella dysenteriae* and *Citrobacter freundii*). A tube containing2 ml of 18 hrs nutrient broth without extract was seeded with a loopful of the test organism previously diluted to 0.5 McFarland turbidiometric standard to serve as the positive control while a tube containing 2 ml of 18 hrs nutrient broth that was not inoculated served as the negative control. After incubation for 24 hours at 37^{o}C, the tubes were then examined for microbial growth by observing the turbidity. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any visible sign of growth and inoculated on sterile nutrient agar by streaking. Nutrient agar plates were streaked with the test organisms only to serve as control. The plates were then incubated at 37^{o}C for 24 hours. After incubation, the concentration at which no visible growth was seen was recorded as the minimum bactericidal concentration.

Statistical analysis

All experiments were carried out in triplicate. Data obtained were analyzed by one way analysis of variance (ANOVA) and means were compared by Duncan's New Multiple Range test using SPSS 15.0 version. Differences were considered significant at $P \leq 0.05$.

RESULTS

The coliform level of water samples is presented in Table 1. The mean counts of faecal coliforms and total coliforms were generally high. The highest count was observed in Shagari stream. The coliforms isolated include *Escherichia coli, Enterobacter aerogenes, Serratia marcescens, Klebsiella pneumonia* and *Citrobacter freundii*. Table 2 shows the percentage yield of extracts obtained. The ethanolic extract had the highest yield of 21.67%. The least recovery was obtained for aqueous extract with 16.67%. Qualitative phytochemical screening showed that the plant extracts contained saponins, tannins, flavonoids and cardiac glycosides, while alkaloids, phlobatannin and anthraquinones were absent. Only aqueous extract did not possess steroids whereas terpenoids were present in aqueous and ethanol extracts of *M. oleifera* (Table 3). The antibacterial activities were shown in Table 4. Aqueous extract did not exhibit broad spectrum antimicrobial activity. The results showed that the test organisms were resistant to the hot water extract except *K. pneumoniae* (6.0 mm) at all concentration and *E. coli* (2.0 mm) at 50 mg/ml to 400 mg/ml. However, all the isolates were sensitive to acetone extracts, ethanol extracts, chloroform and methanol fractions except *E. aerogenes* at 12.5mg/ml in ethanolic extract. Relative to the crude extracts at concentration of 12.5 mg/ml, the chloroform fraction had higher zones of inhibition on all isolates except on *S. dysenteriae* and *S. typhi* in acetone and ethanolic extracts, including *P. vulgaris* in acetone extract while the methanolic fraction had comparatively higher zones of inhibition on *K. pneumoniae, E. aerogenes, C. freundii and P. vulgaris* (ethanolic extract) including *S. marcescens* in acetone

extract. Table 5 showed the minimum inhibitory concentrations which ranged from 4.0 mg/ml to 35.0 mg/ml and the minimum bactericidal concentrations ranged from 5.0 mg/ml to 45.0 mg/ml. There is significant difference between the antibacterial activities of crude and fractionated extracts.

DISCUSSION

The study revealed that the three studied water samples contained coliforms at different levels of concentration. The test plant (*Moringa oleifera*) leaf extracts exhibited antibacterial activities on isolated coliforms from the water samples. Willey *et al*. (2008) reported the isolated microorganisms as major coliforms from water samples. Okonko *et al*. (2008) also reported that the presence of coliforms group in water samples generally suggests a certain portion of water may have been contaminated with faeces either of human or animal origin.

The different percentage recovery and phytochemical analysis observed in studied plant extracts may have resulted from various solvents used in extraction as reported by Kordali *et al*. (2003) and Srinivasan *et al*. (2001) that different solvents have different extraction capacities and different spectrum of solubility for the phytoconstituents respectively. The highest percentage recovery observed in ethanolic extract of *M. oleifera* leaf may be due to the polar bonds present in ethanol which is more active in extracting plants' metabolites. This observation is in agreement with Campos *et al*. (2002) who reported that polar solvents have been shown to be more effective in extracting organic and inorganic materials from plants. The observed results of phytochemical analysis indicated the presence of active constituents in plant leaf extract. This finding was in agreement with previous works of Bukar *et al*. (2010) who reported the presence of flavonoids in *M. oleifera* leaf. The absence of alkaloids in *M. oleifera* extracts had also been reported by Bukar *et al*. (2010). The study of Igbinosa *et al*. (2009) showed that these phyto-constituents are known to be biologically active and therefore aid the antimicrobial activities.

The antimicrobial activity of *M. oleifera* leaf extracts showed different zones of inhibition which were found to be organism and solvent dependent. This may be as a result of observed difference in polarity of bioactive compounds that were extracted. Makanjuola *et al*. (2010) also reported that antibacterial activities can be rationalized in terms of the polarity of the bioactive compounds to diffuse in the culture media used for visible susceptibility reaction by the test organisms to the extracts. The aqueous leaf extract of *M. oleifera* was found to be inactive. This may be because hot water is not efficient enough to extract inhibitory compounds from the plant extract. Caceres *et al*. (1992) reported that the aqueous leaf extract of *M. oleifera* was inactive as antimicrobials which was in line with present study. The observed results showed that the organic extracts (acetone and ethanol) had higher antibacterial activity compared to the aqueous extracts of *M. oleifera* leaf. This is in line with the findings of Koduru *et al*. (2006), Aliero *et al*. (2006), Ashafa *et al* (2008) and Aiyegoro *et al*. (2008) that aqueous extracts of plants generally showed little or no antibacterial activities which were similar to our findings. Cowan (1999) reported that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts.

Antibiotics were more effective in inhibiting test organisms than the plant extracts. This may be due to purity level of commercial antibiotics. This is in line with the findings of Doughari *et al*. (2007) who reported that antibiotics are in a refined state while the plant extracts are still in a crude state. The observed high value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant leaf extracts may be as a result of refined materials used in the production of antibiotics. This is in consonance with Oladunmoye (2005) who reported that antibiotics have high degree of purity; conventional antibiotics and other pharmaceutical products are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures expressing purity and high fractionation which certainly will enhance antimicrobial effect than crude extracts. Most purified extracts had higher inhibitory effect on test organisms than corresponding crude extracts.

Lenta *et al.*(2007) also reported that crude extracts are liable to contamination and deterioration which reduces their in susceptibility activity. This is an indication that when active extracts of *M. oleifera* are purified, they may be potent material for drug development that could be used in the treatment of water borne diseases caused by these coliforms.

CONCLUSION

The study confirmed the presence of coliforms in Shagari stream, Ala River and FUTA stream and the inhibitory effect of *M. oleifera* leaf extracts against isolated coliforms. The ethanolic and acetone extracts were the most effective extracts in this research. Hence, they are the reliable extracts to be used as antimicrobials against diseases caused by the isolated coliforms from surface waters. Further analysis and toxicological study is recommended to be carried out on the effective extracts before assured administration. The availability and accessibility to plants' parts could be an added advantage in the use of *M. oleifera* as an alternative medicine in the treatment of water borne diseases.

Table 1: Coliform level of surface water samples

Surface water sample	Total coliform counts (cfu/ml)±SD	Faecal coliform counts (cfu/ml)±SD
Shagari Stream	$48.59\times10^{3}\pm6.56^{c}$	$83.20\times10^{2}\pm7.64^{c}$
Ala River	$33.51\times10^{3}\pm5.30^{b}$	$41.01\times10^{2}\pm9.02^{b}$
FUTA Stream	$12.81\times10^{3}\pm10.40^{a}$	$39.05\times10^{2}\pm5.51^{a}$

Values are means ±standard deviation for three samples. Mean followed by the same letter(s) within the group are not significantly different at $p\leq0.05$ using Duncan's New Multiple Range test.

Legend: cfu/ml = colony forming unit per milliliter, SD = Standard deviation

Table 2: Percentage recovery of *Moringa oleifera* leaf extracts

Extract	Amount extracted (g)±SD	Percentage recovery (%)±SD
Hot Water	33.34 ± 4.20^{a}	16.67 ± 2.10^{a}
Ethanol	43.24 ± 6.09^{c}	21.62 ± 1.34^{c}
Acetone	35.72 ± 5.23^{b}	17.86 ± 2.16^{b}

Values are means ±standard deviation for three samples. Mean followed by the same letter(s) within the group are not significantly different at $p\leq0.05$ using Duncan's New Multiple Range test.

Legend: SD = Standard deviation

Table 3: Qualitative analysis of phytochemicals of *Moringa oleifera* leaf extracts

Phytochemicals	Aq	Ac	E
Alkaloids	-	-	-
Tannins	+	+	+
Saponins	+	+	+
Phlobatannins	-	-	-
Anthraquinones	-	-	-
Flavonoids	+	+	+
Steroids	-	+	+
Terpenoids	+	-	+
Cardiac Glycosides	+	+	+

Legend: Aq = aqueous, Ac= acetone, E = ethanol, + = positive; - = negative, SD = Standard deviation

Table 4: Antimicrobial activities of *Moringa oleifera* extracts on the isolated organisms (coliforms)

		ZONES OF INHIBITION (mm)±SD							
Extract	Conc. (mg/ml)	SHI	SAL	SER	KLE	ENT	ESC	CIT	PRO
ETHANOL	400	13.10±0.10^{i}	9.22±0.10^{i}	13.03±0.06^{i}	12.10±0.10^{g}	7.10±0.10^{i}	8.07±0.06^{f}	6.17±0.15^{g}	7.10±0.10^{i}
	200	13.05±0.01^{h}	9.21±0.25^{h}	13.01±0.02^{h}	12.02±0.02^{f}	7.03±0.06^{h}	8.01±0.01^{e}	6.05±0.01^{f}	7.03±0.06^{h}
	100	12.20±0.10^{g}	9.00±0.01^{g}	13.00±0.01^{g}	10.01±0.01^{e}	7.01±0.01^{g}	6.13±0.12^{d}	4.01±0.01^{e}	5.12±0.16^{g}
	50	12.03±0.06^{f}	7.10±0.10^{f}	9.06±0.06^{f}	8.06±0.06^{d}	5.01±0.01^{e}	6.03±0.06^{c}	3.03±0.06^{c}	4.03±0.06^{f}
	25	6.10±0.10^{e}	3.13±0.15^{e}	9.04±0.01^{e}	8.01±0.01^{d}	2.01±0.01^{b}	6.01±0.01^{b}	3.02±0.01^{c}	4.01±0.02^{e}
	12.5	6.00±0.01^{d}	3.01±0.01^{d}	5.02±0.02^{c}	3.01±0.01^{a}	0.0^{a}	4.01±0.01^{a}	2.01±0.01^{a}	1.01±0.01^{b}
C2	12.5	3.20±0.10^{c}	1.70±0.05^{a}	6.60±0.20^{d}	3.20±0.10^{b}	5.67±0.59^{f}	11.50±0.05^{g}	3.20±0.05^{d}	2.80±0.05^{d}
M2	12.5	3.00±0.05^{b}	2.00±0.05^{b}	5.00±0.05^{b}	5.00±0.15^{c}	4.40±0.10^{d}	4.00±0.10^{a}	2.90±0.10^{b}	2.60±0.10^{c}
GEN	10(µg)	0.00^{a}	2.33±1.53^{c}	4.33±1.52^{a}	18.67±3.06^{h}	4.00±1.00^{c}	24.67±2.51^{h}	3.00±0.25^{b}	0.00^{a}
ACETONE	400	11.10±0.10^{i}	7.02±0.03^{e}	9.03±0.06^{h}	11.02±0.02^{g}	9.02±0.02^{f}	10.10±0.10^{d}	6.03±0.06^{h}	8.10±0.10^{f}
	200	11.01±0.02^{h}	7.01±0.01^{e}	9.02±0.02^{gh}	11.00±0.01^{f}	9.00±0.01^{f}	10.02±0.02^{d}	6.01±0.01^{g}	8.01±0.02^{e}
	100	11.00±0.01^{g}	4.00±0.01^{d}	9.01±0.01^{g}	9.01±0.02^{e}	6.10±0.10^{e}	10.01±0.01^{d}	5.10±0.10^{f}	8.01±0.01^{e}
	50	9.01±0.01^{f}	4.00±0.00^{d}	5.10±0.10^{e}	9.01±0.01^{e}	6.02±0.02^{d}	8.10±0.10^{c}	5.07±0.12^{e}	4.01±0.01^{d}
	25	6.06±0.07^{e}	2.01±0.02^{b}	5.03±0.06^{d}	7.10±0.10^{d}	6.00±0.01^{d}	8.01±0.02^{c}	5.01±0.01^{d}	3.02±0.02^{d}
	12.5	5.01±0.01^{d}	2.00±0.01^{b}	3.03±0.06^{a}	3.01±0.01^{a}	4.01±0.01^{a}	7.01±0.01^{b}	2.00±0.01^{a}	3.01±0.10^{d}
C2	12.5	3.20±0.10^{c}	1.70±0.05^{a}	6.60±0.20^{f}	3.20±0.10^{b}	5.67±0.59^{c}	11.50±0.05^{e}	3.20±0.05^{c}	2.80±0.05^{c}
M2	12.5	3.00±0.05^{b}	2.00±0.05^{b}	5.00±0.05^{c}	5.00±015^{c}	4.40±0.10^{b}	4.00±0.10^{a}	2.90±0.10^{b}	2.60±0.10^{b}
GEN	10(µg)	0.00^{a}	2.33±1.53^{c}	4.33±1.52^{b}	18.67±3.06	4.00±1.00^{a}	24.67±2.51^{f}	3.00±0.25^{b}	0.00^{a}
	400	0.0^{a}	0.0^{a}	0.0^{a}	6.01±0.02^{f}	0.0^{a}	2.01±0.01^{e}	0.0^{a}	0.0^{a}
AQUEOUS									
	200	0.0^{a}	0.0^{a}	0.0^{a}	6.00±0.01^{f}	0.0^{a}	1.64±0.01^{d}	0.0^{a}	0.0^{a}
	100	0.0^{a}	0.0^{a}	0.0^{a}	5.99±0.04^{e}	0.0^{a}	1.25±0.01^{c}	0.0^{a}	0.0^{a}
	50	0.0^{a}	0.0^{a}	0.0^{a}	5.99±0.01^{e}	0.0^{a}	1.24±0.01^{b}	0.0^{a}	0.0^{a}
	25	0.0^{a}	0.0^{a}	0.0^{a}	4.01±0.02^{c}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
	12.5	0.0^{a}	0.0^{a}	0.0^{a}	2.02±0.02^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
C2	12.5	3.20±0.10^{c}	1.70±0.05^{b}	6.60±0.20^{d}	3.20±0.10^{b}	5.67±0.59^{d}	11.50±0.05^{g}	3.20±0.05^{c}	2.80±0.05^{c}
M2	12.5	3.00±0.05^{b}	2.00±0.05^{c}	5.00±0.05^{c}	5.00±0.15^{d}	4.40±0.10^{c}	4.00±0.10^{f}	2.90±0.10^{b}	2.60±0.10^{b}
GEN	10(µg)	0.00^{a}	2.33±1.53^{d}	4.33±1.52^{b}	18.67±3.06^{g}	4.00±1.00^{b}	24.67±2.51^{h}	3.00±0.25^{b}	0.00^{a}

Mean followed by the same letter(s) within the group are not significantly different at $p \leq 0.05$ using Duncan's New Multiple Range test.
Legend: SHI = *Shigella dysenteriae*, SAL = *Salmonella typhi*, SER = *Serratia marcescens*, KLE = *Klebsiella pneumoniae*, ENT = *Enterobacter aerogenes*, ESC = *Escherichia coli*, CIT = *Citrobacter freundii*, PRO = *Proteus vulgari*, SD= Standard deviation, C2 = chloroform fraction, M2 =methanol fraction, GEN = Gentamycin, SD = Standard deviation

Table 5: Minimum inhibitory concentration (mg/ml) and minimum bactericidal concentration (mg/ml) of *Moringa oleifera* leaf extracts

	MIC			MBC		
Organisms	Hot Water	Acetone	Ethanol	Hot Water	Acetone	Ethanol
SHI	ND	7.5	4.0	ND	8.0	5.0
SAL	ND	10.0	8.0	ND	10.0	8.0
SER	ND	8.0	5.0	ND	10.0	9.0
KLE	11.0	7.0	10.0	11.5	7.0	11.0
ENT	ND	6.0	ND	ND	6.0	ND
ESC	35.0	4.5	4.0	45.0	5.0	8.0
CIT	ND	10.0	10.0	ND	11.0	12.0
PRO	ND	8.0	10.0	ND	9.0	12.0

Legend: SHI = *Shigella dysenteriae*, SAL = *Salmonella typhi*, SER = *Serratia marcescens*, KLE = *Klebsiella pneumoniae*, ENT = *Enterobacter aerogenes*, ESC = *Escherichia coli*, CIT = *Citrobacter freundii*, PRO = *Proteus vulgaris*, ND = Not Determined, MIC = Minimum inhibitory concentration, MBC = Minimum bactericidal concentration

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