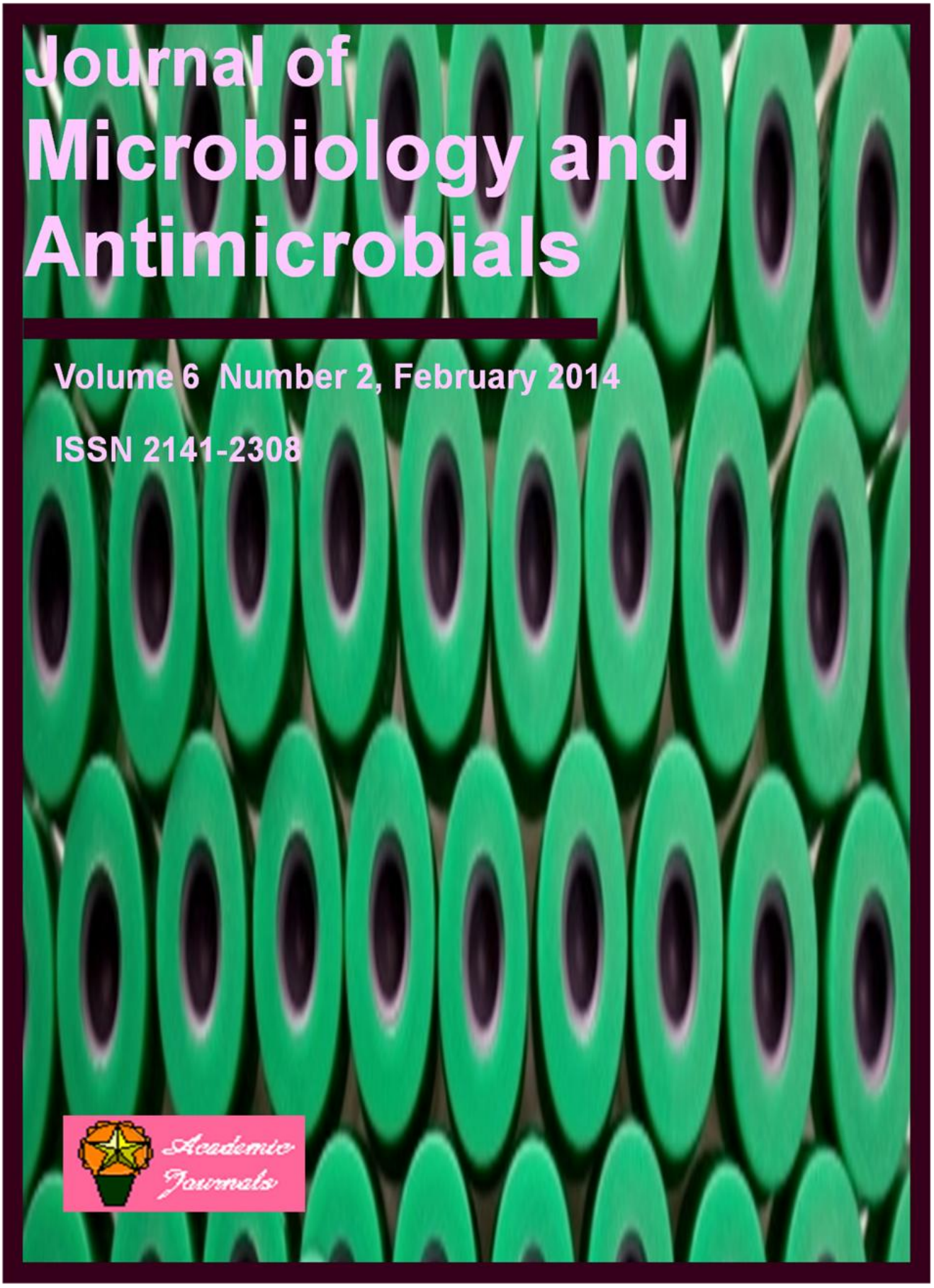
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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Antimicrobial activities of blinding tree, *Excoecaria agallocha* against selected bacterial pathogens

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In aquaculture, the occurrence of bacterial resistance to synthetic antibiotics has become a serious problem. Therefore, research has been focused on finding new antimicrobial antibiotics from natural products to replace synthetic antibiotics. The aim of this study was to investigate the antimicrobial properties of mangrove plant blinding tree *Excoecaria agallocha* against selected fish pathogens namely *Flavobacterium indicum*, *Chryseobacterium indologenes*, *Chryseobacterium gleum* and *Elizabethkingia meningoseptica* previously named *Flavobacterium meningosepticum*. Mangrove leaves were obtained via extraction with 100 ml of methanol. The antimicrobial susceptibility test showed that the bacteria were resistant to Nitrofurantion, Gentamycin and Neomycin, and were sensitive to Flumequine. The minimum inhibitory concentration (MIC) of *E. agallocha* was 3.12 mg/ml, and minimum bactericidal concentration (MBC) was 6.25 mg/ml. Inhibition zones were significantly different ($p< 0.05$) depending on concentrations (100, 300 and 500 mg/ml) of the crude extraction of *E. agallocha*. The highest activity with LC_{50} of *E. agallocha* was 94.19 (mg/ml). Methanolic extract of *E. agallocha* exhibited strong antimicrobial activity against these bacteria.

Key words: Antimicrobial activities, *Excoecaria agallocha*, inhibition zone, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), LC_{50}.

INTRODUCTION

Antimicrobial agents have been widely used in aquaculture worldwide to treat infections caused by a variety of fish bacterial pathogens. Excessive use of antimicrobial agents in aquaculture has led to antimicrobial resistance among bacteria including those that are not fish pathogens. Many studies have been carried out on the plant antimicrobial properties against aquatic bacterial pathogens (Lee et al., 2008; Najiah et al., 2011a; Najiah et al., 2011b). Laith (2012) reported the presence of antimicrobial activities of mangrove plants such as *Sonneratia caseolaris* and *Rhizophora apiculata* on fish pathogenic bacteria. *Excoecaria agallocha* latex is well known to cause skin irritants, rapid blistering, and temporary blindness in human. This latex has been used as a poison for fish by adding it to water as well as to poison arrowheads. The plant is also used to treat flatulence (Karalai et al., 1994). Mangrove contains biologically active antibacterial, anti-fungal and antiviral compounds (Vadlapudi et al., 2009). Mangrove is a natural resource of tannin and is of great value; however, the antioxidant and antifungal potentials against fish pathogen is limited (Dhayanithi et al., 2012). Nowadays, interest in the plant extract of *E. agallocha* bark herbal preparation due to its antimicrobial activities is increasing. Plant extract has been suggested as beneficial in the healing of severe infections, in addition to anti-tumour, anti-microbial, anti-wound killing and anti-oxidant properties (Thirunavukkarasu et al., 2009). The antibacterial activity of methanolic extract of 10 plants

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species was carried out *in vitro* by disc diffusion method against 10 both Gram-positive and Gram-negative bacteria. The antibacterial results revealed three levels of activities; maximum, moderate and minimum activity on bacteria, this might be as a result of the presence of flavanoids, alkaloids, phenolic and glycosides compounds in the plant extracts (Gothandam et al., 2010). The value of these secondary metabolites is increasing due to the constant discoveries of their potential role in health care and drug development (Remani et al., 2012).

Flavobacteria are found in soil and water, several species are identified in causing diseases in fish such as *Flavobacterium psychrophilum* in salmond and rainbow trout fry- a disease known as bacterial cold water disease. *Flavobacterium columnare* was reported to cause cotton-wool disease in freshwater fishes (Bernardet et al., 1994).

The *Chryseopbacterium* genus was a member of the Flavobacteriaceae such as *Chryseopbacterium meningosepticum*, *Chryseopbacterium indologenes*, *Chryseopbacterium indoltheticum*, *Chryseopbacterium scophthalmum* and *Chryseopbacterium gleum* (Vandamme et al., 1994). *C. meningosepticum* and *C. miricola* have been reclassified into the new genus *Elizabethkingia* (Kim et al., 2005). In the study of Montasser (2005), isolation of *C. meningosepticum* from chicken and tick, *Argas persicus* was performed. *E. meningoseptica* is a significant bacterial group in human clinical infections. Recently, *E. meningoseptica* was isolated from tiger frogs, *Rana tigerina rugulosa* with cataract disease in China (Xie et al., 2009). *Chryseobacterium* species, including *C. indologenes* and *C. meningosepticum*, have been documented as human pathogens in hospitalized patients implanted with indwelling devices (Hsueh et al., 1996; Hsueh et al., 1997). *C. indologenes* is responsible mostly for nosocomial infections linked to the use of intravascular devices (Hsueh et al., 1996). *Chryseobacterium* spp. is known to exhibit resistance to aminoglycosides, tetracyclines, chloramphenicol and erythromycin (Kim et al., 2005). *C. indologenes* appears to be an emerging problem in Taiwan because of its multi-resistance to antibiotics (Hsueh et al., 1997).

Therefore, the aim of the present study was to determine the antimicrobial activity of methanolic extracts from leaves of the mangrove plant blinding tree, *E. agallocha* against selected bacterial pathogens.

MATERIALS AND METHODS

Bacterial stock

Fish bacterial stock was obtained from Fish Disease Laboratory, University Malaysia Terengganu (UMT) Malaysia. They were *Flavobacterium indicum*, *Chryseobacterium indologenes, Chryseobacterium gleum and Elizabethkingia meningoseptica* (*Chryseobacterium* spp, *Elizabethkingia* spp, were previously put under Flavobacterium family).

Plant material

E. agallocha leaves were collected from the mangrove rural area in Terengganu, Malaysia. The plant was identified at the Plant Taxonomy Laboratory, University Malaysia Terengganu (UMT), Malaysia. The weight of *E. agallocha* leaves after extraction was determined and the percent yield of the leaves was calculated using the formula (Sule et al., 2011):

Percentage yield = $X_1 - (X_2 / X_1) \times 100.0\%$

Where, X_1 = Weight before extraction; X_2 = Weight after extraction.

Extract preparation

The plant leaves were washed with tap water and then with distilled water to remove epiphytes and other debris. The leaves were then air dried in a shaded area for 3 weeks before being grounded to powder (15 g dry weight) and then extracted with 100 ml of methanol for 48 h according to the methods of Bele et al. (2009). The extract was filtered using a filter paper. This procedure was repeated three times and the samples were pooled. The solvent was evaporated from the crude extract by a rotator evaporator (Buchi, Switzerland) and the dried extracts were stored at 4°C until further use. The percentage yield was calculated from the dry extract powder. Then, 3,250 mg/ml of the extracts were dissolved in methanol for the antibacterial assays and in dimethyl sulfoxide (DMSO) for the cytotoxicity assays. The samples for the cytotoxicity screening were further diluted to 5 mg/ml with growth medium (DMEM or RPMI 1640) to reduce the concentration of DMSO.

Antibiotics susceptibilities tests (disk diffusion method)

Antibiotic susceptibility was determined following the methods as described by Bauer et al. (1966). Isolates were tested *in vitro* for their sensitivity to 18 different antibiotics namely; Oxolinic Acid (2 µg), Flumequine (30 µg) Deoxycycline (30 µg), Ampicillin (10 µg), Oleandomycin (15 µg), Fosfomycin (50 µg), Nitrofurantion (50 µg), Spiramycin (100 µg), Lincomycin(15 µg), Neomycin (10 µg), Tetracycline (10 µg), Florfenicol (30 µg), Amoxicillin (25 µg), Erythromycin (15 µg), Colistin Sulphate (25 µg), Novobiomycin (30 µg), Gentamycin (10 µg) and Polymyxin B (30 µg) (Oxoid, England).

The isolates in the current study were cultured in Anacker and Ordal's broth (EAOB) at 28°C for 48 h. Minispin tube (Eppendorf, Germany) was used to centrifuge the bacterial cells at 14,500 rpm for 5 min. Bacterial cell concentrations were adjusted to McFarland 0.5 ($1.5 \times 10^8 CFUmL^{-1}$). 100 µl of aliquots were spread over Anacker and Ordal's agar (EAOA) surface using sterile cotton buds. After a time lapse of 10 min, antibiotic disks were placed on the surface of the inoculated agar plates using sterile forceps. The plates were incubated at 28°C for 48 h. After incubation, the diameter of inhibition zones around the discs were measured in millimetre (mm) and characterized as sensitive (S), intermediate (I) and resistant (R) according to Clinical and Laboratory Standard Institute (CLSI, 2006).

Antibacterial assay

Disk diffusion assay

Disk diffusion assay was carried out on Anacker and Ordal's agar

(EAOA) following the method described by Barker et al. (1995). Briefly, the disc (6 mm in diameter) was impregnated with 10 mg/ml extract (20 µg/disc) and placed on inoculated agar. Antibiotic disc was used as positive control (Flumequine 30 µg/disc) and discs impregnated with (20 µl) of methanol was used as negative controls. Discs were then air dried and placed equidistantly onto the EAOA agar surface layered with bacterial pathogens and incubated at 28°C for 48 h. The growth inhibition was assessed as the diameter (mm) of the inhibition zone around the discs. The experiment was carried out in triplicate.

Agar-well diffusion method

Anacker and Ordal's broth (EAOB) was used to grow bacterial isolates for 48 h prior to use. The concentration of the suspensions was adjusted to achieve a turbidity of 0.5 McFarland 1.5×10^8 $CFUmL^{-1}$. Sterilized cotton swabs were used to seed isolates on Anacker and Ordal's agar (EAOA). A hole in the wells of the agar medium was made by means of a sterilized 6 mm cork borer. Wells were later filled with 100 µl solution of various concentrations of extracts (100, 300 and 500 mg/well) and 100 µl of methanol (negative control) was dispensed into separate wells. The standard antibiotic disc flumequine (30 µg) was left to set on the agar surface as positive control. The plates were laid inside an incubator at 28° C for 48 h (Perez et al., 1990). Following that, plates were inspected for zone of inhibition. The experiment was carried out in triplicate.

Determination of minimum inhibitory concentration (MIC)

The MIC of plants crude extracts was determined for active component that showed antimicrobial activity against test organisms. The micro titre broth dilution technique was performed, according to standards methods, using sterile 96 well-micro titre plates (CLSI, 2006). Bacteria were cultured overnight in Anacker and Ordal's broth (EAOB) at 28°C and adjusted to achieve a turbidity of 0.5 McFarland $1.5 \times 10^8 CFUmL^{-1}$. A 100 (µl) of Anacker and Ordal's broth (EAOB) were allocated to each wells. For each assay, the first wells were inoculate with 100 (µl) of 100 (mg/ml) crude extracts, followed by the two fold dilution until 0.098 mg/ml. Microbial suspension of 10µl were used as inoculants. Positive growth control included broth and inoculums (without extracts suspension). Micro titre plates were sealed with parafilm to ensure the bacteria does not become dehydrated. Plates were further incubated at 28°C for 48 h. After incubation, turbidity of each wells was observed visually and the optical density (OD) were measured at 540 nm by a micro-titre reader model 680 (Bio-rad, US). Data dilution values lower than the yielding value ≥ 2, the doubling concentrations were interpreted as MIC results. The results were confirmed with micro dilution assays with the addition of 10 µl of 0.1% 2,3,5-triphenyltetrazolium chloride (TTC) (w/v) (Merck, Germany) into each well and incubated for 1 h for reaction. Colour changes from purple to pink were observed visually where the bacteria were able to reduce the TTC into formazon. The lowest concentrations that inhibit the growth of bacteria with the absence of visual colour changes were recorded as MIC. Tests were run in triplicate.

Determination of Minimum bactericidal concentrations (MBC)

MBC extract was determined according to Ilavenil et al. (2010). Plates that displayed negative results from the MIC assay were taken as samples and sub-cultured onto a new Anacker and Ordal's agar (EAOA); they were incubated at 28°C for 48 h. The MBC inhibited bacterial growth on agar plate surface and therefore was the lowest concentration of extract. Experiments were performed in triplicate of three and the mean readings were recorded. The ratio of MBC /MIC determined the results. If ratio of MBC/MIC was ≤ 2, the active crude extract was considered as bactericidal; if not, it was considered as bacteriostatic. If ratio was ≥16, then the active crude extract is considered to be ineffective (Shanmughapriya et al., 2008).

Brine shrimp lethality test

The experiment was carried out according to a previously described method (Pisutthanan et al., 2004). Brine shrimp eggs (OSI, USA) were hatched in sea water using a 1,000 ml beaker and then incubated at room temperature (28°C) for 24 h. Nauplii were collected using 100 µl tips after removing 2 mm from the tip ends. One hundred milligrams of the extracts were dissolved in 10% dimethyl sulphoxide (DMSO) (Chempur, US). 2-fold serial dilutions were made in 96-well micro-plates using 100 µl of sea water in triplicate. A suspension of nauplii containing 10-15 organisms (100 µl) was added to each well and incubated at room temperature (28°C) for 24 h. The control sample contained 10% DMSO without extracts. The plates were then examined under a binocular stereomicroscope (Nikon, Japan) and the number of dead nauplii in each well was counted. One hundred microlitres of methanol were added to each well to immobilise the nauplii and the total numbers of nauplii were taken after 15 min. The data analysis was performed using the linear regression of probit method to determine the lethality of LC_{50} value.

Statistical analysis

Data were expressed as mean ± standard deviation of triplicate measurements. Probit linear regression analysis was done to analyse the LC_{50} values using statistical package for the social sciences (SPSS) version 16.0 for windows. Analysis of variance (ANOVA) and the mean was compared with least significant difference ($P<0.05$) using Gestate 12.1 program.

RESULTS

Antibiotic resistance test was done on all selected samples of Gram negative bacteria. Isolates showed 100% resistant to Nitrofurantion, Gentamycin and Neomycin and, 100% sensitive to Flumequine (Table 1). The percent yield was 7.0% and the mass of the crude was 0.15 g. Antimicrobial activities of methanolic extract of *E. agallocha* on bacteria were carried out by measuring inhibition zone, using agar well and disc diffusion methods.

The mean zone of inhibition for *E. agallocha* methanol extracts of concentration 100, 300 and 500 (mg/ml) using well technique ranged from 15.67 to 20.33, 18.67 to 20 and 18.67 to 23.67 mm, respectively. When the mean zone of inhibition against all isolates for the flumequine antibiotic ranged from 25.19 to 27.33 mm, there was a significant difference ($P<0.05$) among all concentration and antibiotic against bacteria (Table 2).

The result revealed there are significant difference ($P<0.05$) as compared to the mean inhibition zone for *E. agallocha* methanol extracts of concentration 100, 300 and 500 (mg/ml). Although, the concentration of 500 mg/ml showed the high inhibition zone than 100 and 300

Table 1. Percentage (%) of antibiotic resistance (R), intermediately sensitive (I) and sensitive (S) of the present isolate.

Antibiotic	Disc potency (mcg)	Isolates					Zone of inhibition			
		*1	*2	*3	*4	*5	R	I	S	S (%)
Ampicillin	10	S	R	R	R	R	14	15-16	17	20
Flumequine	30	S	S	S	S	S	15	16-18	21	100
Oleandomycin	15	S	R	R	R	R	12	13-16	17	20
Fosfomycin	50	S	R	R	R	R	13	14-16	17	20
Nitrofurantion	50	I	R	R	R	R	19	20-25	26	0
Spiramycin	100	S	R	R	R	R	12	13-15	16	20
Lincomycin	15	S	R	R	R	R	14	15-20	21	20
Neomycin	10	R	R	R	R	R	12	13-15	17	0
Tetracycline	10	S	R	R	R	R	14	15-18	19	20
Florfenicol	30	S	R	R	R	S	14	15-17	18	40
Amoxicillin	25	S	R	R	R	R	13	14-17	18	20
Deoxycycline	30	S	S	S	R	R	14	15-18	19	60
Erythromycin	15	S	R	R	I	R	13	14-22	23	20
Colistin sulphate	25	S	R	R	R	R	8	9-10	11	20
Novobiomycin	30	I	I	S	I	R	17	18-21	22	20
Oxolinic acid	2	S	S	S	I	R	14	15-17	18	60
Gentamycin	10	R	R	R	R	R	12	13-14	15	0
Polymyxin B	30	S	R	R	R	R	8	9-11	12	20

1(*F. indicum*), 2 (*E. meningoseptica* (kidney), 3 (*E. meningoseptica*(skin), 4 (*C. gleum*), 5 (*C. indologenes*).

Table 2. Comparison of antimicrobial activities of crude methanolic extract of *E. agallocha* on bacterial growth.

Bacteria	Concentration						
	100 mg/ml	300 mg/ml	500 mg/ml	Flumequine		Methanol	
C. gleum	15.67^{fg}	18.67^{cde}	23.33^{b}	26.67	a	0	h
C. indologenes	16.67^{def}	18.67^{cde}	18.67^{cde}	25.19	a	0	h
E. meningosepticum (kidney)	18.67^{cde}	20.33^{c}	23.33^{b}	25.67	a	0	h
E. meningosepticum (skin)	20.33^{c}	18.67^{cde}	23.67^{b}	27.33	a	0	h
F. indicum	14.00^{g}	14.00^{g}	16.33^{efg}	27.33	a	0	h
L.S.D (least significant difference)			2.265				

Different letter show significant differences ($p < 0.05$).

mg/ml concentration but the result revealed there are significant difference ($P<0.05$) as compared to the mean inhibition zone of flumequine (Figure 1).

The present result was confirmed by using disc diffusion assay and showed significant difference ($P<0.05$) in compareson with antibiotics Flumequine and disc of *E. agallocha* methanol extract against individual test bacteria (Figure 2).

The antimicrobial activity of the extracts was quantitatively assessed by determining the MIC and MBC, respectively. The lowest MIC and MBC values for the *E. agallocha* methanol extracts on test bacteria were 3.12 and 6.25 mg/ml (Table 3).

The toxicity of brine shrimp was done dependently on the mortality data from concentration of 50 to 0.08 (mg/ml). It was analyzed by SPSS version 16.0 to obtain the 50% lethal concentration (LC_{50}). The LC_{50} value was calculated by probit method which showed 94.19 mg/ml (Figure 3).

DISSCUSION

Antimicrobial susceptibility data on *Chryseobacterium* spp. remain very limited, since this pathogen has been rarely isolated from clinical specimens (Fraser and Jorgensen, 1997). *Chryseobacterium* spp. is recognized to demonstrate resistance towards aminoglycosides,

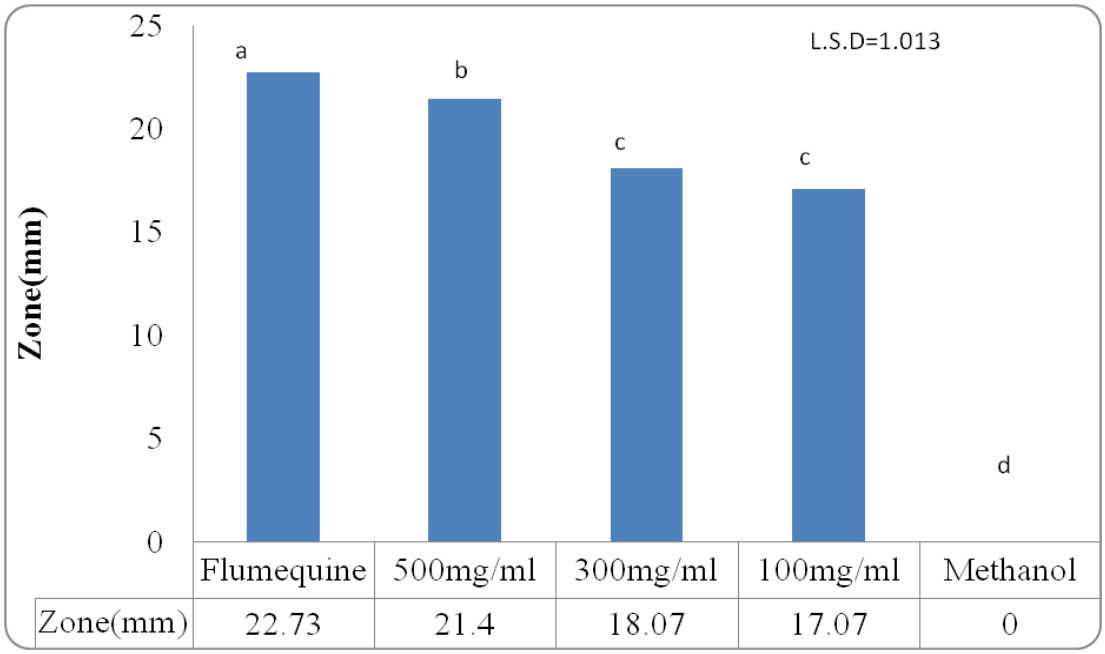

Figure 1. Mean inhibition zone of different concentration of crude methanolic extract of *E. agallocha* and antibiotic. Different letter show significant differences ($p<0.05$).

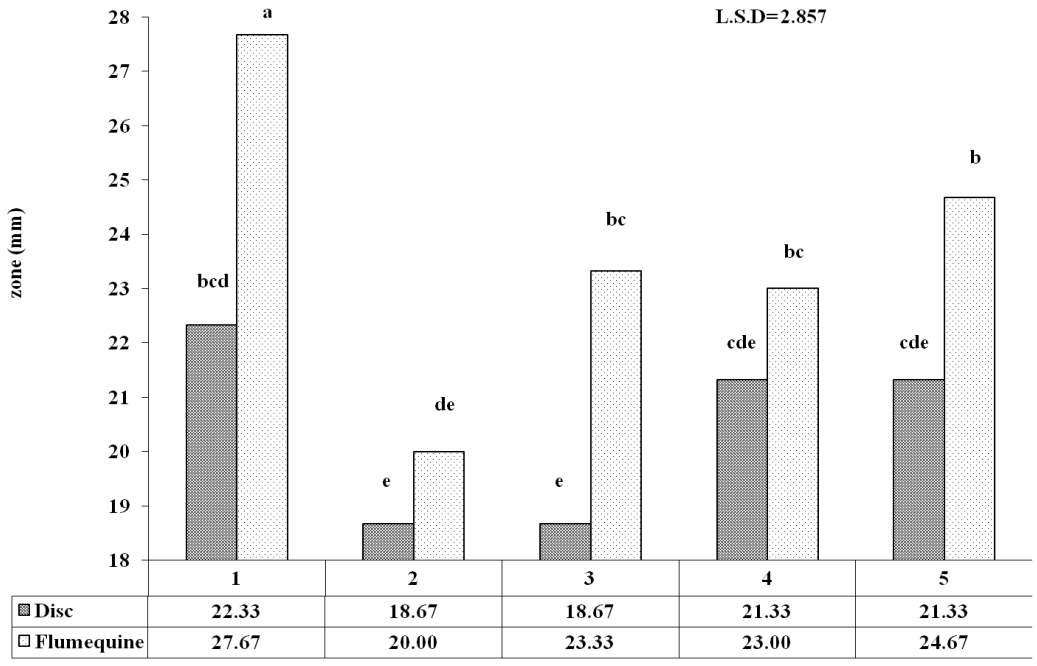

Figure 2. Comparison of disc and antibiotics using Disc Diffusion Assay on the mean inhibition zone of bacterial group. 1 (*F. indicum*), 2 (*E. meningoseptica* (kidney), 3 (*E. meningoseptica* (skin), 4 (*C. gleum*), 5 (*C. indologenes*). Different letters show significant differences ($p<0.05$).

tetracyclines, erythromycin, and chloramphenicol (Hsueh et al., 1997). Therefore, we used this type of bacteria in our study. In another study, Chang (1997) reported more than 90% of *Flavobacterium* spp. isolates showed resistance to amino-glycosides, glycopeptides and macrolides. However, the authors suggested for treating infections

Table 3. MIC, MBC values of active extract on test bacteria (mg/ml).

Bacteria	MIC	MBC	MBC/MIC
F. indicum	6.25[a]	6.25[a]	1.05[b]
E. meningoseptica (kidney)	6.25[a]	6.25[a]	0.99[b]
E. meningoseptica (skin)	6.25[a]	6.33[a]	1.01[b]
C. gleum	6.25[a]	6.25[a]	1.05[b]
C. indologenes	3.12[b]	6.25[a]	2.00[a]
L.S.D (least significant difference)	0.45	0.47	0.12

Different litter show significant differences ($p<0.05$).

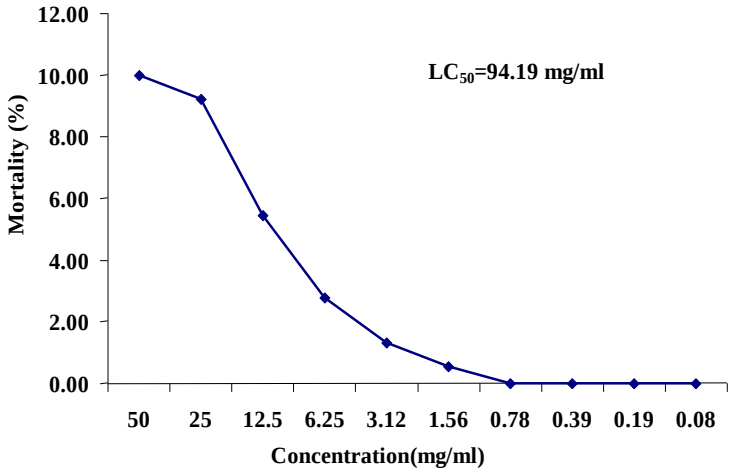

Figure 3. Brine shrimp lethality of *E. agallocha* against *A. salina.*

caused by *Chryseobacterium* spp, that the determination of the MIC for each individual isolate is mandatory.

The antimicrobial susceptibility test performed in our experiment showed that all isolates appeared to be resistant to Nitrofurantion, Gentamycin and Neomycin and sensitive to Flumequine. Although, majority of isolates demonstrated a high level of bacterial resistance towards antibiotics in the present study this finding is in agreement with Maraki (2009) indicating similarities among isolates might be due to the close genetic relatedness.

Products come from a new source of antibacterial agents becoming popular in covering the basic health needs against infective microorganisms. In the meantime, applications of herbs as biomedicine are becoming more important in disease treatment in aquaculture sectors (Citarasu et al., 2010). Mangroves are wide spread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coast lines. They have also been found to contain biologically active antiviral, antibacterial and antifungal compounds (Ravikumar et al., 2009). *Excoecaria agallocha* extract is gaining enormous interest as an alternative to antibiotics. Although, the result from present study showed Flumequine antibiotic had the highest significant effect on bacteria growth which was formally used as drug of choice in the treatment of infection caused by *Flavobacterium* spp. (Sano et al., 1998) but still the value of inhibition zone of *E. agallocha* methanolic extract at concentration 500 (mg/ml) was more closely effective to the flumequine antibiotic.

C. meningosepticum was usually resistant to multiple antibiotics. Otherwise, it was susceptible to fluoroquinolones and trimethoprim-sulfamethoxazole, andvancomycin (Ozkalay et al., 2006). These resistance phenoltypes could be explained by the presence of beta-lactamases, including extended-spectrum beta-lactamases and metallo-beta-lactamases (Vessillier et al., 2002). Previous research on *E. meningoseptica* reported was highly resistant to aminoglycosides, tetracyclines, chloramphenicol, erythromycin, clindamycin and teicoplanin (Hoque et al., 2001; Tekerekoglu et al., 2003; Lin et al., 2004).The antimicrobial resistance mechanisms of *Chryseobacterium* species are still unknown. Efflux pump systems have been established in many bacteria that have shown multiple drug resistances. These systems are accountable for the active and nonspecific removal of foreign substances from the cell, which includes antimicrobial substances (Michel et al., 2005).

Based on disc diffusion results, the antimicrobial activity

of *E. agallocha* methanolic leaves crude extract are in agreement with the result of Suryati and Hala et al. (2002) that the biological activity test of mangrove *E. agallocha* crude extract against *Vibrio mimicus* showed inhibitory activity of 10.35 ± 0.05 mm at 10 µL and 12.6 ± 0.05 mm at 20 µL using diffusion agar methods. Our finding is compatible with previous report of Kumar (2009) who reported that mangrove *E. agallocha* leaf extracts showed maximum inhibitory activity of 18 mm against *Streptoccocus aureus.* The diameters of the zone of inhibition shown by *E. agallocha* methanolic leaves crude extract against the pathogenic bacteria are similar to the study of Chandrasekaran (2006). The present results revealed that the *E. agallocha* minimal inhibition concentration (MIC) value was 3.12 mg/ml against bacteria. This finding are in accordance with the previous study of Patra (2009) who recorded the MIC of *E. agallocha* methanolic leaves crude extract to range from 5 to 7 mg/ml against the following bacteria; *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis*, *Staphylococcus aureus, Vibrio cholera, Shigella flexneri*, *Bacillus licheniformis, Bacillus brevis* and *Escherichia coli.*

Nowadays, demand on medicinal plants has increased for new drugs due to the bacterial resistance to present drug. The low toxicity of medicinal plant play an important role in new drugs. Therefore, brine shrimp larvae have been used as a bioassay for a variety of toxic substances (Ameen et al., 2011). Based on previous study, the brine shrimp assay is a simple and useful tool for the isolation of potentially cytotoxic compounds from plant extracts (Meyer et al., 1982). The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials. Our results are in agreement with study of Nusrat (2008) which claimed that the extract of mangrove plant *E. agallocha* showed considerable brine shrimp toxicity (LC_{50} = 20 mg/mL), and the variation in the BSLA results may be due to the difference in the amount and kind of cytotoxic substances such as; tannins, flavonoids or triterpenoids present in the extracts.

Recently, study on the mechanism of antimicrobial activity of plant against *Vibrio cholerae* (Sanchez et al., 2010) and *Vibrio parahemolyticus* and *Vibrio alginolyticus* (Nadirah et al., 2013) concluded that the mechanism of extracts of edible and medicinal plants occurs through the changes in membrane integrity, membrane potential, internal pH and ATP synthesis of cell bacteria and cause damage to the membrane of Vibrios exerting profound physiological changes that lead to bacterial death. Another research done by (Yamasaki et al., 2011) concluded that the potential of natural compounds may have direct inhibition on virulence gene expression in *Vibrio cholerae*. Furthermore, this inhibitory mechanism may be inhibited by the secreted cholera toxin (CT) or the growth of bacteria.

The present results are in accordance with the results of previous research that *E. agallocha* extracts are recognized for their antimicrobial activities (Subhan et al., 2008; Ravikumar et al., 2009; Thirunavukkarasu et al., 2011). The bioactivity properties of *E. agallocha* might be due to presence of a higher relative percentage of antibacterial substances. On the other hand, the present results are in accordance with the results of Agoramoorthy et al. (2007) and Ravikumar et al. (2009) that *E. agallocha* leaf extract display antimicrobial activity.

Conclusion

Based on the screening results, *E. agallocha* has showed promising antibacterial potential to combat the fish pathogenic bacteria. Determination of bioactive compound of the mangrove plants will be carried out in the near future.

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REFERENCES

Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind your eye mangrove from India. Braz. J. Microbiol. 38:739-742.

Ameen OM, Olatunji GA, Atata RF, Usman LA (2011). Antimicrobial activity, cytotoxic test and phytochemical screening of extracts of the stem of *Fadogia agrestis*. Niseb J. 11:79-84.

Barker GA, Kehoe E (1995). Assessment of disc diffusion methods for susceptibility testing of *Aeromonas salmonicida* . Aquacult. J. 134:1-8.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standard B single disc method. Am. J. Clin. Pathol. 45:493-496.

Bele AA, Jadhav VM, Nikam SR, Kadam VJ (2009). Antibacterial potential of herbal formulation. Res. J. Microbiol. 4(4):164-167.

Bernardet JF, Kerouault B, Michel C (1994). Comparative study on *Flexibacter maritimus* strains isolated from farmed sea bass (*Dicentrarchus labrax*) in France. Fish Pathol. 29:105-111.

Chandrasekaran M, Kannathasan K, Venkatesalu V, Sivasankari S, Sajitkhan AK, Rajendran S, Prabhakar K (2006). Antibacterial activity of salt certain mangrove plants against methicillin resistant *Staphylococcus aureus*. Seaweed Researchers and Utilization 28:165-170.

Chang JC, Hsueh PR, Wu JJ, Ho SW, Hsieh WC, Luh KT (1997). Antimicrobial susceptibility of flavobacteria determined by agar dilution and disk diffusion methods. Antimicrob. Agents Chemother. 41:1301-1306.

Citarasu T (2010). Herbal biomedicines: a new opportunity for aquacultue industry. Aquacult. Int. 18:403-414.

CLSI (2006). Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Sixteenth Informational Supplement CLSI, Wayne, PA, USA, 26:M100-S116.

Dhayanithi NB, Ajith Kumar TT, Balasubramanian T (2012). Effects of *Excoecaria agallocha* leaves against *Aeromonas hydrophila* in marine ornamental fish, *Amphiprion sebae*. Indian J. Geo-Mar. Sci. 4(1):76-82.

Fraser SL, Jorgensen JH (1997). Reappraisal of the antimicrobial susceptibilities of *Chryseobacterium* and *Flavobacterium* species and methods for reliable susceptibility testing. Antimicrob. Agents Chemother. 41:2738-2741.

Gothandam KM, Aishwarya R, Karthikeyan S (2010). Preliminary screening of antimicrobial properties of few medicinal plants. J. Phytol. 2:01-06.

Hoque SN, Graham J, Kaufmann ME, Tabaqchali S (2001). *Chryseobacterium* (*Flavobacterium*) *meningosepticum* outbreak associated with colonization of water taps in a neonatal intensive care unit. J. Hosp. Infect. 47:188-192.

Hsueh PR, Hsiue TR, Hsieh WC (1996). Pyomyositis in intravenous drug abusers: report of a unique case and review of the literature. Clin. Infect. Dis. 22:858-860.

Hsueh PR, Teng LJ, Yang PC, Ho SW, Hsieh WC and Luh KT (1997). Increasing incidence of nosocomial *Chryseobacterium indologenes* infections in Taiwan. Eur. J. Clin. Microbiol. Infect. Dis. 16:568-574.

Karalai C, Wiriyachitra P, Opferkuch HJ, Hecker E (1994). Cryptic and free skin irritants of the daphnane and tigliane in latex of *Excoecaria agallocha*. Planta Med. 60:351-355.

Kim KK, Kim MK, Lim JH, Park HY, Lee ST (2005). Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov.as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. Int. J. Syst. Evol. Microbiol. 55:1287-1293.

Kumar R, Ramanathan S, Subhakaran M, Jacob S, Inbaneson G (2009). Antimicrobial compounds from marine halophytes for silkworm disease treatment. Int. J. Med. Med. Sci. 1:184-191.

Laith AA, Najiah M, Zain SM, Effendy SHM, Tee AW, Nadirah M, Habsah M (2012). Antimicrobial Activites of Selected Mangrove Plants on Fish Pathogenic Bacteria. J. Anim. Vet. Adv. 11:234-240.

Lee SW, Najiah M, Chuah TS, Wendy W, Noor AMS (2008). Antimicrobial properties of trobical plants against 12 pathogenic bacteria isolated from aquatic organisms. Afr. J. Biotechnol. 7:2257-2278.

Lin PY, Chu C, Su LH, Huang CT, Chang WY, Chiu CH (2004). Clinical and microbiological analysis of bloodstream infections caused by *Chryseobacterium meningosepticum* in nonneonatal patients. J. Clin. Microbiol. 42:3353-3355.

Ilavenil SB, Kaleeswaran B, Ravikumar S (2010). Evaluation of antibacterial activity and phytochemical analysis of *Crinum asiaticum.* Int. J. Curr. Res. 1:035-040.

Maraki S, Scoulica E, Manoura A, Papageorgiou N, Giannakopoulou C, Galanakis E (2009). A *Chryseobacterium meningosepticum* colonization outbreak in a neonatal intensive care unit. Eur. J. Clin. Microbiol. Infect. Dis. 28:1415-1419.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, MacLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents, Planta Med. 45:31-34.

Michel C, Matte-Tailliez O, Kerouault B and Bernardet JF (2005). Resistance pattern and assessment of phenicol agents' minimum inhibitory concentration in multiple drug resistant *Chryseobacterium* isolates from fish and aquatic habitats. J. Appl. Microbiol. 99:323-332.

Montasser AA (2005). Gram-negative bacteria from the camel tick *Hyalommadromedarii* (Ixodidae) and the chicken tick *Argaspersicus* (Argasidae) and their antibiotic sensitivities. J. Egypt Soc. Parasitol. 35:95-106.

Nadirah M, Wee TL, Najiah M (2013). Differential responses of Vibrio sp. to young and mature leaves extract of Terminalia catappa L. Int. Food Res. J. 20:961-962.

Najiah M, Nadirah M, Laith AA, Ibrahim WN, Zaroul MA, Arief ZI, Ranzi MA, Wee TL, Mariam M, Amar S, Awang S (2011a). Methanolic Activities of Selected Weeds on Bacteria Isolated from Macrobrachium rosenbergii Larvae. Thai J Vet Med. 41. 535-539.

Najiah M, Nadirah M, Laith AA, Arief Z, Zahrol S, Tee LW, Ranzi AD, Amar S, Maryam M, Suzana S, Aida RJ (2011b). Antibacterial Activity of Malaysian Edible Herbs extracts on fish pathogenic bacteria. Res. J. Med. Plant 5:722-778.

Nusrat S, Mohammad A, Firoj A, Israt JSL, Satyajit NDS (2008). Bioactivity of *Excoecaria agallocha*. Braz. J. Pharmacogn. 18:521-526.

Ozkalay N, Anil M, Agus N, Helvaci M, Sirti S (2006). Community-acquired meningitis and sepsis caused by *Chryseobacterium meningosepticum* in a patient diagnosed with thalassemia major. J. Clin. Microbiol. 44:3037-3039.

Patra KT, Panigrahi TK, Rath SK, Dha NK, Thatoi H (2009). Phytochemical screening and antimicrobial assessment of leaf extracts of *Excoecaria agallochal*.: A Mangal Species of Bhitarkanika, Orissa, India Adv. Nat. Appl. Sci. 3:241-246.

Perez C, Paul M, Bazerque P (1990). An Antibiotic assay by the agar well diffusion method. Acta. Bio. Med. Exp. 15:113-115.

Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S, Muanrit O (2004). Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae. Naresuan Univ. J. 12:13-18.

Remani KN, Jalaja TK, Asish GR (2010). Antimicrobial activity of wetland plant *Excoecaria Agallocha* on *Escherichia Coli* and *Streptococcus Haemolyticus*. The IUP J. Life Sci. 4(1):42-47.

Ravikumar S, Ramanathan G, Subhakaran M (2009). Antimicrobial compounds from marine halophytes for silkworm disease treatment. Int. J. Med. Med. Sci. 1:184-191.

Sanchez E, García S, Heredia N (2010). Extracts of edible and medicinal plants damage membranes of *Vibrio cholerae*. Appl. Environ. Microbiol. 76: 6888-6894.

Sano T (1998). Control of fish disease, and the use of drugs and vaccines in Japan. J. Appl. Ichthyol 14:131-137.

Shamsuddin AA, Najiah M,Suvik A, Azariyah MN, Kamaruzzaman BY, Effendy AW, Akbar John B (2013). Antibacterial properties of selected mangrove plants against Vibrio species and its cytotoxicity against artemia salina. World Appl. Sci. J. 25:333-340.

Shanmughapriya S, Manilal A, Sujith S, Selvin J, Kiran G, Natarajaseenivasan K (2008). Antimicrobial activity of seaweeds extracts against multiresistant pathogens. Ann. Microbiol. 58:535-541.

Subhan N, Alam MA, Ahmed F, Shahid IJ, Nahar L, Sarker SD (2008). Bioactivity of *Excoecaria agallocha*. Revista Brasileira de Farmacognosia 18:521-526.

Sule WF, Okonko IO, Omo-Ogun S, Nwanze JC, Ojezele MO, Ojezele OJ, Alli JA, Soyemi ET and Olaonipekun TO (2011). Phytochemical properties and *in-vitro* antifungal activity of *Senna alata* Linn. Crude stem bark extract. J. Med.Plants Res. 5:176-183.

Suryati E, Hala Y (2002). Bioactive substances of mangrove *Exoecaria agallocha* as shrimp diseases inhibitor. Mar. Chim. Acta 2:9-14.

Tekerekoglu MS, Durmaz R, Ayan M, Cizmeci Z, Akinci A (2003). Analysis of an outbreak due to *Chryseobacterium meningosepticum* in a neonatal intensive care unit. The new microbiologica 26:57-63.

Thirunavukkarasu SV, Lokesh U, Venkataraman S (2011). Effect of aluminum induced toxicity on behavioral and hematological parameters under the influence of manasamitra vatakam (an ayurvedic formulation) in rats. Pharmacol. online 1:594-603.

Vadlapudi V, Naidu KC (2009). In vitro antimicrobial activity *Ceriops Decandra* against Selected Aquatic, Human and Phytopathogens. Int. J. Chem. Tech. Res. 4:1236-1238.

Vandamme P, Bernardet JF, Segers P, Kersters K, Holmes B (1994) New perspectives in the classification of the flavobacteria: Description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Enpedobacter* nom. rev. Int. J. Syst. Bacteriol. 44:827-831.

Vessillier S, Docquier JD, Rival S, Frere JM, Galleni M, Amicosante G, Rossolini GM, Franceschini N (2002). Overproduction and biochemical characterization of the *Chryseobacterium meningosepticum* BlaB Metallo-β-Lactamase. Antimicrob. Agents Chemother. 46:1921-1927.

Xie ZY, Zhou YC, W SF, Bing M, Xu XD, Wen WY, Feng YQ (2009). First isolation and identification of *Elizabethkingia meningoseptica* from cultured tiger frog, *Rana tigerina rugulosa*. Vet. Microbiol. 138:140-144.

Yamasaki S, Asakura M, Neogi SB, Hinenoya A, Iwaoka E, Aoki S (2011). Inhibition of virulence potential of *Vibrio cholerae* by natural compounds. Indian J. Med. Res. pp.133-232.

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Full Length Research Paper

Assessment of bacteriological contaminants of some vegetables irrigated with Awash River water in selected farms around Adama town, Ethiopia

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Food safety issues are of growing concern to consumers globally because of the risk associated with consumption of foods contaminated with pathogens in irrigated vegetables. The study was conducted to assess the extent of bacterial contamination of vegetables due to irrigation with polluted Awash River water. Three leafy vegetable samples, namely, cabbage (*Brassica oleracea L. var. capitata*), lettuce (*Lactuca sativa L. longifolia*) and spinach (*Spinacea oleracea*) from both farms were examined for bacterial contaminants. The results show that spinach was found to be the most heavily contaminated vegetable in both farms by aerobic bacteria. The aerobic mesophilic bacterial count on this vegetable was 2.2×10^8 and 2.0×10^8 CFU/g, for spinach sampled from Melka Hida and Wonji Gefersa vegetable farms, respectively. The highest total coliform count (6.6×10^6) was also recorded from lettuce in Melka Hida vegetable farm. The mean fecal coliform values of all the three vegetable samples exceed the International Commission on Microbiological Specifications for Foods (ICMSF) recommended level. The highest faecal coliform count (5.7×10^5) was recorded in cabbage sampled from Wonji Gefersa. The high microbial contamination rates associated with these vegetable samples indicated poor water quality for irrigation employed in the overall production of vegetables in the study area.

Key words: Indicator bacteria, pathogen, vegetables.

INTRODUCTION

Food safety is a major public health concern worldwide. During the last decades, the increasing demand on food safety has stimulated research regarding the risks associated with consumption of food stuffs contaminated with pathogenic microorganism. Several studies have revealed that contamination of vegetables with pathogens poses a threat for consumers (D'Mello, 2003; Zandstra and De Kryger, 2007). Vegetables are produced in significant quantities both in urban and pre urban areas.

The United Nations Development Program estimated in 1996 that more than 800 million people were engaged in urban agriculture globally. Of these people, about 200 million practice market-oriented farming on open spaces, often using poor quality irrigation water. As urban populations in developing countries increase, and residents seek better living standards, larger amounts of freshwater

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are consumed by domestic, commercial and industrial sectors, which generate greater volumes of wastewater (Qadir et al., 2007a; Asano et al., 2007).

In addition to these, waste and sewage water are still considered as the richest sources of nutrients for plants. In many cities and towns, the sewage water is sold and it is a good source of income to municipalities (Scott et al., 2004). However, the situation is changed now with the establishment of industries in suburban areas where the wastewater is mixed with industrial effluents and big culverts are coming out from the cities. The polluted water is still used for growing vegetables in the nearby areas of the cities without knowing their adverse impacts on the life of consumers (Qadir, 1999). Furthermore farmers, consumers, and some government agencies in many countries are not fully aware of the potential impacts of irrigation with wastewater (Qadir et al., 2007b; Amoah, 2009).

The access to clean water for irrigating vegetables is a major challenge. Consequently, urban and peri-urban vegetable farmers have no other choice than to use water from these highly polluted sources. This raises public health concerns due to possible crop contamination with pathogens where vegetables are eaten uncooked (Amoah et al., 2006). In developing countries, continued use of untreated wastewater and manure as fertilizers for the production of vegetables is a major contributing factor to contamination that causes numerous foodborne disease outbreaks (Johannessen et al., 2002; AdeOluwa, and Cofie, 2012).

Adama and Wonji Gefersa Towns are found in Adama *Woreda* of East Showa zone, Oromia Region, with a population of 260,600 and 23,510, respectively (CSAE, 2005). Awash River is the only important river in Adama *Woreda* used for irrigating about1132 ha of land. Awash River flows from central highlands through Ethiopia's major industrial and agro-industrial belt, taking in a whole burden of all types of raw effluent stands as one of Ethiopia's river streams in urban areas of developed rivers (Tesfamariam, 1989). Besides this, the expansion of new industries and disposal of industrial wastes to the Awash River basin is of great concern to the nation (Girma Taddese, 2001). Application in farming of such untreated waste can pose significant health hazard to those who have direct contact with it and eventually the public becomes affected through the food chain link (Furedy et al., 1999).

Information on the microbial safety of food items in Ethiopia is limited. Many farm households around Adama Town that are irrigating their farmlands with Awash River are not aware of the risks or the potential harmful environmental consequences. This may be attributed to illiteracy, lack of adequate information and exposure to poor sanitary conditions for most of their lives. Altogether, the situation puts the consumers at high risk of contracting diseases. The magnitude of microbial contaminants in vegetables grown on irrigated with Awash River is not known in the study area. Therefore, the study was designed to assess the levels of indicator bacteria on these vegetables irrigated with wastewater in Melka Hida and Wonji Gefersa farms around Adama Town.

MATERIALS AND METHODS

Description of the study area

This study was conducted at two wastewater irrigated vegetable growing farms, that is, Melka Hida and Wonji Gefersa that are found in Adama *Woreda*. Melka Hida is found in Adama Town Administrative Zone, Oromia Region, which is 99 km away from Addis Ababa and is located at latitude of 8° 33′ 0″ North and longitude 39° 16′ 12″ East. It has an elevation of 1620 m above sea level.

Wonji Gefersa is a town that is found in Adama *Woreda* of East Showa Zone, Oromia Region, nearby Adama Town, which is 107 km away from Addis Ababa and located at a latitude of 8° 26' 59'' North and longitude of 39° 16' 48'' East. It has an elevation of 1588 m above sea level and its temperature and annual rain fall is 23°C and 500-800 mm, respectively (Environmental Protection Authority, 2005). Awash River is the only important river in Adama *Woreda* used for irrigating around 1132 ha of land, which originates from the highlands of Dandi *Woreda* located west of Addis Ababa, Ethiopia, and flows along the rift valley into the Afar region, where it eventually terminates in a salty lake, Lake Abbe, found on the border with Djibout (Figure 1)

Study design

A cross sectional survey was conducted to assess the load of bacterial contamination on the main leafy vegetables [lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea Linn*.), and spinach (*Spinacea oleracea*)] that were grown in Melka Hida and Wonji Gefersa vegetable farms irrigated with wastewater. The samples were regularly collected at three week interval during February 2012 and April 2012, and analyzed for aerobic mesophilic bacterial count, feacal coliform count and total coliforms.

Sample collection

A total of 72 samples comprising three types of fresh vegetables (cabbage, lettuce, spinach,) were collected from Melka Hida and Wonji Gefersa vegetable farms using a random sampling technique method. Recently, mature leaves of lettuce, cabbage and spinach were sampled at early maturity according to methods used by Fisseha (1998). All samples were collected aseptically in a sterilized universal container and plastic bags and transported to Haramaya University for laboratory processing. The samples were cooled during transportation using a cooler box to keep the normal conditions of the microflora of vegetables. The analysis began immediately after the sample arrival at laboratory.

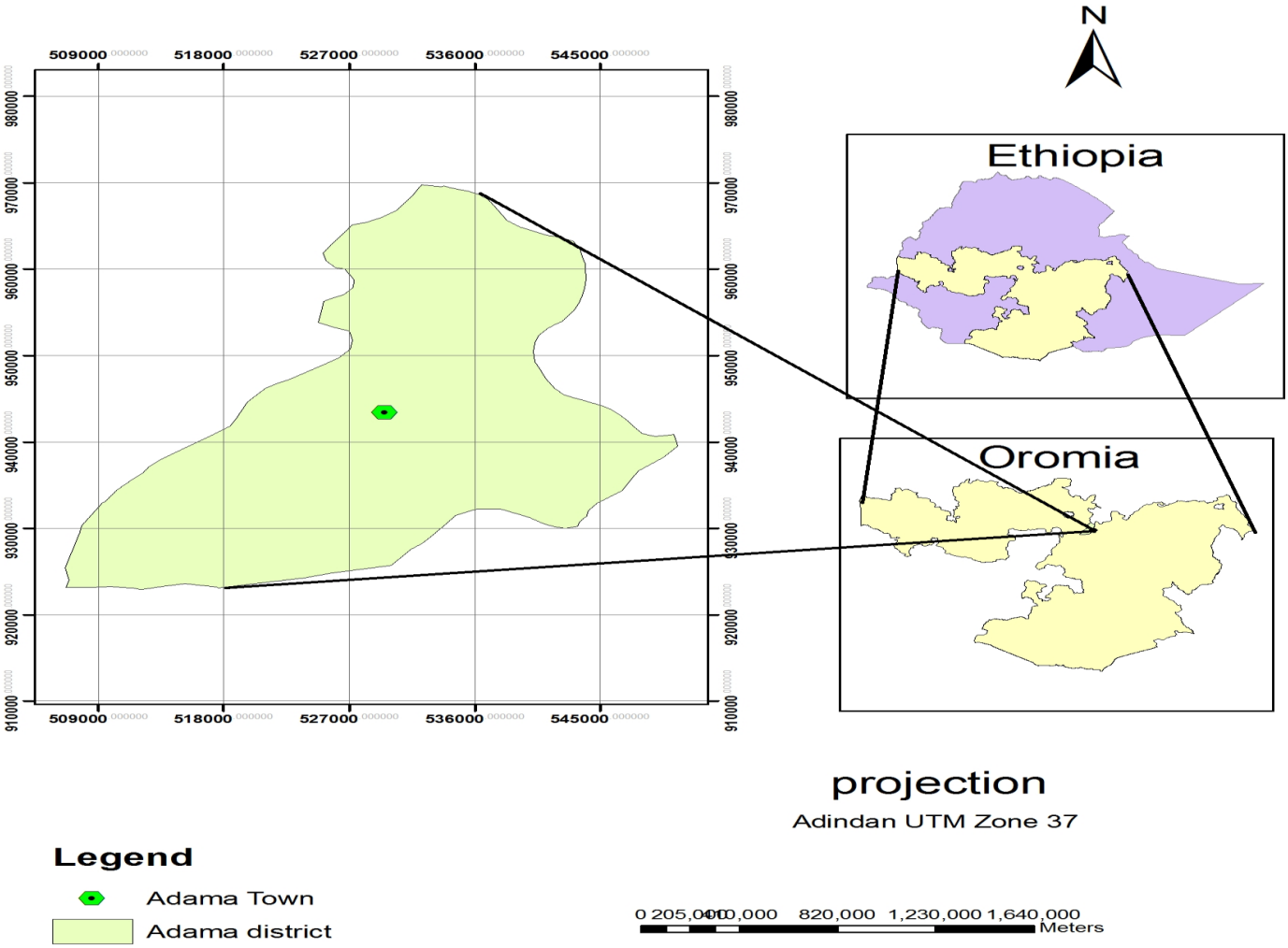

Figure 1. Location of the study area. Source: Aynalem Adugna (2010).

Microbiological analysis

For microbiological analysis, 25 g of leafy material was aseptically removed from each vegetable sample using a sterile scalpel and blended in 225 ml of sterile 0.1% (w/v) bacteriological peptone water for 1-3 min. The samples were homogenized using stomacher (Seward Stomacher, Model: 400 Circulator, Rate frequency: 50-60Hz). The homogenate was used as a source of microbial source for determining the aerobic mesophilic bacterial counts, total coliform counts and feacal coliform counts following the standard methods of APHA (1998).

Data analysis

In this study, all statistical analyses were computed using SAS software version 9.1 for microbiological analyses. The data were subjected to analysis of variance (ANOVA) to assess the effect of Vegetable type and site of production on the concentrations of microbial contaminants in the vegetables tested. As the level of microbial contamination might vary with sample collection site and vegetable type, ANOVA was used to test the existence of significant difference between means. In all statistical analyses, confidence level was held at 95% and $P<0.05$ (at 5% level of significance) was considered as significant.

RESULTS AND DISCUSSION

Bacteriological analysis of leafy vegetables

This study attempted to determine the percentage of vegetable contamination with aerobic mesophilic bacteria (AMB), coliform bacteria (TCC) and faecal coliforms (FC) as well as their microbial loads through aerobic mesophilic bacterial counts (AMB), total coliform counts (TCC) and fecal coliforms (FC). The highest percentage was obtained

Table 1. Percentage of positive samples of indicator bacteria in spinach, lettuce and cabbage samples from both farms.

Site	Indicator organism	No. of sample examined	Type of plant sample					
			Spinach		Lettuce		Cabbage	
			F	%	F	%	F	%
Melka Hida	AMB	12	12	100	12	100	12	100
	TCC	12	12	100	10	83.3	10	83.3
	FC	12	8	66.6	7	58.3	9	75
Wonji Gefersa	AMB	12	12	100	12	100	12	100
	TCC	12	12	100	11	91.6	10	83.3
	FC	12	9	75	8	66.6	7	58.3

F= Frequency of positive samples, AMB = aerobic mesophilic bacteria, TCC = total coliform count, FC = faecal coliforms.

Table 2. Mean number of indicator bacteria among the tested vegetable samples of Melka Hida and Wonji Gefersa.

No. of examined samples	Number of indicator organisms (CFU/g)	Site	Vegetable types			
			Cabbage	Lettuce	Spinach	Mean
12	AMB	MH	$9.3 \times 10^7 \pm 8.8^c$	$1.7 \times 10^8 \pm 8.8^b$	$2.2 \times 10^8 \pm 8.8^a$	$1.6 \times 10^8 \pm 5.1$
12		WG	$8.0 \times 10^7 \pm 8.8^c$	$1.6 \times 10^8 \pm 8.8^b$	$2.0 \times 10^8 \pm 0.8^a$	$1.5 \times 10^8 \pm 5.1$
		Mean	$8.7 \times 10^7 \pm 6.2$	$1.6 \times 10^8 \pm 6.2$	$2.1 \times 10^8 \pm 6.2$	$1.6 \times 10^8 \pm 3.1$
12	TCC	MH	$5.0 \times 10^6 \pm 0.8$	$6.6 \times 10^6 \pm 0.8$	$5.2 \times 10^6 \pm 0.8$	$5.6 \times 10^6 \pm 0.5^d$
12		WG	$4.1 \times 10^6 \pm 0.8$	$4.1 \times 10^6 \pm 0.8$	$3.1 \times 10^6 \pm 0.8$	$3.8 \times 10^6 \pm 0.5^e$
		Mean	$4.5 \times 10^6 \pm 0.6$	$5.4 \times 10^6 \pm 0.6$	$4.1 \times 10^6 \pm 0.6$	$3.7 \times 10^6 \pm 0.3$
12	FC	MH	$5.2 \times 10^5 \pm 0.7^a$	$3.1 \times 10^5 \pm 0.7^b$	$3.7 \times 10^5 \pm 0.7^b$	$4.0 \times 10^5 \pm 0.4$
12		WG	$5.7 \times 10^5 \pm 0.7^a$	$2.3 \times 10^5 \pm 0.7^b$	$2.2 \times 10^5 \pm 0.7^b$	$3.4 \times 10^5 \pm 0.4$
		Mean	$5.5 \times 10^5 \pm 0.5$	$2.7 \times 10^5 \pm 0.5$	$3.0 \times 10^5 \pm 0.5$	$3.7 \times 10^5 \pm 0.2$

$^{a\text{-}b\text{-}c}$ Means with different superscript letters along the row for the same parameter in the same site do significantly differ ($P<0.05$); $^{d\text{-}e}$ means with different superscript letters along the column for the same parameter do significantly differ ($P<0.05$).

for aerobic mesophilic bacteria (100%) as demonstrated by its occurrence in all vegetable samples analyzed. On the other hand, 100, 83.3, 83.3 and 100, 91.6, 83.3% of the samples of spinach, lettuce and cabbage collected from Melka Hida and Wonji Gefersa, respectively, were found contaminated with coliform bacteria. Similarly, fecal coliforms were found in 66.6, 58.3, 75, and 75, 66.6, 58.3% of spinach, lettuce and cabbage collected from Melka Hida and Wonji Gefersa farms, respectively (Table 1).

From the data in Table 1, it can be understood that there was an improper pre harvest handling of the vegetables in selected study area. The high percentage of vegetables contaminated with coliform bacteria and fecal coliforms may suggest high risk of acquiring infectious diseases through the consumption of these vegetables. The occurrence of such indicator microorganisms is an indication of the contamination of the vegetables with faecal matter derived from humans and other animals (Cornish et al., 1999).

The results of this investigation additionally showed that the vegetable samples collected from both farms (Melka Hida and Wonji Gefersa) were heavily contaminated by aerobic mesophilic bacterial counts ranging from $8.0 \times 10^7 \pm 8.8$ to $2.2 \times 10^8 \pm 8.8$ CFU/g. The mean values of aerobic mesophilic bacterial counts were in the order of spinach > lettuce > cabbage for both sites as shown in Table 2. The data further showed that all the bacterial counts recorded in this study exceeded the recommended levels by WHO and International Commission on Microbiological Specifications for Food (ICMSF) standards (10 to 10^2 coliforms g^{-1}, 10 faecal coliform g^{-1} and 4.9×10^6 aerobic count g^{-1}) wet weight vegetables.

The results of the analysis of variance for bacterial counts

between locations (Melka Hida and Wonji Gefersa) and vegetables (spinach, lettuce and cabbage) are shown in Table 2. The data showed that there was a highly significant difference ($p<0.01$) in the average counts of AMB amongst the vegetable types, but there was no significant difference ($p>0.05$) between sites. On the other hand with respect to the mean TCCs, the ANOVA did not show significant difference ($p>0.05$) amongst vegetable types, but there was significant difference ($p<0.05$) between sites. However, as can be seen from the same tables, there was a significant difference between the two sites. Similarly, the results of the analysis of variance for FC counts showed that in both farms, there was a significant difference amongst vegetable types but not between sites.

The AMB for spinach, lettuce and cabbage were in the range of 2.0×10^8 - 2.2×10^8, 1.6×10^8 - 1.7×10^8 and 8×10^7 - 9.3×10^7 CFU/g, respectively. This high aerobic mesophilic bacterial count might be due to pollution by humans, animals or irrigation water. In agreement with this result, Thunberg et al. (2004), reported total viable count as 5.0×10^8, 4.0×10^8, 3.1×10^7, 2.5×10^7 and 2.0×10^6 CFU/ g for spinach samples collected from various farm sites. However, spinach collected from both farms (Melka Hida and Wonji Gefersa) accounts a very high aerobic mesophilic bacterial count in terms of CFU/g. The high aerobic mesophilic bacterial counts in spinach could be due to the wide surface area of vegetable leaves which is suitable for water contact and microbial contamination (Anonymus, 2002). The mean aerobic mesophilic bacterial counts of the lettuces in this study is 1.7×10^8 CFU/g. In agreement with this result, Viswanatha and Kaur (2001) from in India indicated that total aerobic plate count for cabbage and lettuce was found to be 2.8×10^6 - 1.2×10^8 and 1.3×10^7 - 2.3×10^7CFU/g, respectively.

The total coliform levels recorded under this study were high in all the three vegetable samples analyzed (Table 2). Total coliform levels ranged from 4.1×10^6 - 5.0×10^6 CFU/g for cabbage, 4.1×10^6 -6.6×10^6 CFU/g for lettuce and 3.1×10^6 -5.2×10^6 CFU/g for spinach at both sites. High total coliform counts (6.6×10^6 CFU/g) were observed on lettuce collected from Melka Hida as compared to the other two vegetable samples. Similar findings are reported by Nguz et al. (2005) in Zambia which found a range of total coliform counts on vegetable products between 1.6×10^2 and 7.9×10^5 CFU/g. According to Nguz et al. (2005), fecal coliform counts are efficient indicators of sanitization, but the presence of fecal coliforms does not necessarily indicate the presence of a pathogen. In this study, the fecal coliform counts of vegetable samples (cabbage, lettuce and spinach) collected from both sites ranged between 5.2×10^5 to 5.7×10^5, 2.3×10^5 to 3.1×10^5 and 2.2×10^5 to 3.7×10^5 CFU/g, respectively. The mean fecal coliform values of all the three vegetable samples exceed the ICMSF recommended level of 10 fecal coliform g^{-1} fresh weights. This may be due to Awash River used for irrigation of vegetables, which flows from central highlands through Ethiopia's major industrial and agro-industrial belt, taking in a whole burden of all types of raw effluent. In addition to this, application of organic manures is common practices of farmer for production of crops in that area.

However, sources of fecal coliform contamination of lettuce may include overhead irrigation of lettuce with already contaminated water, planting in contaminated soils and frequent application of poultry manure which was not well composted (Amoah et al., 2005). Of the three vegetables, cabbage shows significant difference ($p<0.05$) among crop types of both farms by contamination of fecal coliform counts. This shows cabbage was more contaminated by fecal coliforms than other leafy vegetables.

These results correlate with the probability of the vegetable samples to be more in contact with the source of contamination during growth (Heaton and Jones, 2008). Moreover, application of fresh poultry manure without sufficient drying used for vegetable production registers high fecal coliform counts (Drechsel et al., 2000). Generally, variation in the AMB, TCC and FC values of the present study and previous works may be either due to differences in the geographical location of the cultivation area, or due to the difference in contamination load at different sections of the drainage canal and different pre-harvest handling practices.

Conclusion

The study revealed that there was bacterial contamination of fresh leafy vegetables (lettuce, cabbage and spinach) grown in Melka Hida and Wonji Gefersa vegetable farms. Bacterial numbers recorded in this study range from 2.2×10^5 to 2.22×10^8CFU g^{-1} which is above the ICMSF (1998) limit of 10^3 to 10^5 coliforms $100g^{-1}$ wet weight of vegetables usually eaten raw. Spinach was found to be the most contaminated vegetable by aerobic mesophilic bacterial count (2.03×10^8 to 2.22×10^8, CFU/ g). This might be due to the fact that spinach have wide leaves surface in contact with wastewater, soil and dust. In contrast cabbage was the least in aerobic mesophilic bacterial count (8×10^7 to 9.3×10^7 CFU/g).

In this study, high total coliform counts (6.6×10^6 CFU/g) were observed on lettuce collected from Melka Hida among the three vegetable. Likewise, the fecal coliform counts of vegetable samples collected from both site ranged between 2.2×10^5 and 5.7×10^5 CFU/g. However, cabbage showed high fecal coliform counts among the three analyzed vegetable samples. Therefore, great attention should be paid in using contaminated water for production of vegetables for the public health perspective.

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REFERENCES

AdeOluwa OO, Cofie O (2012). Urine as an alternative fertilizer in agriculture: effects in amaranths (Amaranthus caudatus) production in Nigeria. Renew. Agric. Food Syst. 27(4):287-294.

Amoah P, Drechsel P, Abaidoo C (2005). Irrigated urban vegetables production in Ghana: Sources of pathogen contamination and health risk elimination. Irrig. Drainage 54:49-61.

Amoah P, Drechsel P, Abaidoo RC (2006). Pesticide and microbiological contamination of vegetables in Ghana's urban markets. Arch. Environ. Contam. Toxicol (AECT) 50:1-6.

Amoah P (2009). An analysis of the quality of wastewater used to irrigate vegetables in Accra, Kumasi and Tamale, Ghana. In Redwood, M. (Ed.). Agriculture in urban planning: generating livelihood and food security. London, UK: Earthscan. pp. 105-124.

Anonymous (2002). Risk profile on the microbiological contamination of fruits and vegetables eaten raw. European commission health and consumer protection directorate-general SCF/CS/FMH/SURF/Final. Brussels.

APHA (American Public Health Association) (1998). Standard methods for the examination of water and wastewater, 20th Edition, Washington DC.

Asano T, Burton FL, Leverenz HL, Tsuchihashi R, Tchobanoglous G (2007). Water reuse: issues, technologies, and applications. McGraw-Hill, New York. pp. 345-350.

Aynalem Adugna. http://www.ethiodemographyandhealth.or.html. Data Aug 1-2010

Cornish GA, Mensah E, Ghesquière P (1999). Water Quality and Peri-urban Irrigation. An assessment of surface water quality for irrigation and its implications for human health in the Peri-urban Zone of Kumasi, Ghana. report OD/TN 95. HR Wallingford Ltd, Wallingford, UK.

CSAE (Central Statistical Agency of Ethiopia), (2005). Census preliminary pdf-file.

D'Mello JPF (2003). Food safety: Contaminants and toxins. CABI Publishing, Wallingford, Oxon, UK,Cambridge, MA. p. 480

Drechsel P, Abaidoo RC, Amoah P, Cofie OO (2000). Increasing use of poultry manure in and around Kumasi, Ghana: Is farmers' race consumers' fate? Urban Agric. Mag. 2:25-27.

Fisseha I (1998). Metal concentrations of some vegetables irrigated with industrial liquid waste at Akaki, Ethiopia. Ethiop. J. Sci. 21(1):133-144.

Furedy C, Maclare V, Whitney J (1999). Reuse of waste for food Production in Asian Cities: Health Econ. Perspect. pp.136-144.

Girma T (2001). Land degradation: A challenge to Ethiopia. Environ. Manag. 27:815-824.

Heaton JC, Jones K (2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. J. Appl. Microbiol. 104:613-626.

ICMSF (International Commission on Microbiological Specification for Foods) (1998). Microorganisms in foods, vol. 6, Microbial Ecology of food commodities, New York; Blackie Academics and Professional.

Johannessen GS, Loncarevic S, Kruse H (2002). Bacteriological analysis of fresh produce in Norway. Int. J. Food Microbiol. 77:199-204.

Nguz K, Shindano J, Samapundo S, Huyghebaghebaert A (2005). Microbiological evaluation of fresh-cut organic vegetables produced in Zambia. J. Food Contr. 16:623-628.

Qadir M (1999). City people eating poisoned vegetables. The daily dawn. Pakistan. Natl. News paper, Dec.22.

Qadir M, Wichelns D, Raschid-Sally L, Minhas PS, Drechsel P, Bahri A, McCornick P (2007a). Agricultural use of marginal quality water opportunities and challenges. Comprehensive assessment of water. Management in Agriculture. Mc Grow Hill, New York 3:567-570.

Qadir M, Wichelns D, Raschid -Sally L, Minhas PS, Drechsel P, Bahri A, McCornick P (2007b). Agricultural use of marginal-quality water opportunities and challenges. In: Molden, D. (Ed.), Mc Graw Hill, New York pp. 97-99.

Scott C, Faruqui N, Raschid-Sally L (2004). Wastewater use in irrigated agriculture: Confronting the livelihood and environmental realities, pp.193.

Tesfamariam T (1989). Water pollution and natural resources degradation. A challenge to Ethiopia .Beyen D (ed) , First Natural Resources Conservation Conference,7-8 February ,1989, IAR, Addis Ababa.

Thunberg RL, Tran TT, Bennett RW, MatthewsRN and Belay N (2004). Microbial evaluation of selected fresh produce obtained at retail markets. J. food Prot. 65(4):677-682.

Viswanatha P, Kaur R (2001). Prevalence and growth of pathogens on salad vegetables, fruits and sprout. Int. J. Hyg. Environ. Health. 203(3):205-213.

Zandstra BH, De Kryger TA (2007). Arsenic and lead residues in carrots from foliar applications of monosodium methanearosonate (MSMA): A comparison between mineral and organic soils, or from soil residues. Food Addit. Contam. 24:34-42.

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Full Length Research Paper

Antimicrobial susceptibility and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) typing of Gram negative bacteria isolated from urinary tract infections in Mansoura, Egypt

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The aim of the study was to determine the resistance patterns of Gram negative bacterial isolates recovered from patients suffering from urinary tract infections (UTIs) in Mansoura university hospitals, Egypt and also to investigate their epidemiological relatedness using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique. The most prevalent etiological agents of UTIs were the Gram-negative bacilli bacteria including *Escherichia coli*, *Pseudomonas* spp. and *Klebsiella* spp. Among the isolates, *Pseudomonas* spp. showed the highest antimicrobial resistance rate and was significantly resistant to most of the antimicrobials more than other isolates. Antimicrobial susceptibility testing showed that imipenem could be considered as the drug of choice for the treatment of infections caused by multi-resistant isolates of UTIs. SDS-PAGE classified the *E .coli*, *Pseudomonas* and *Klebsiella* isolates into 5, 2 and 5 types, respectively.

Key words: Gram negative bacteria, urinary tract infections (UTIs), antimicrobial susceptibility testing, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

INTRODUCTION

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year (Naish and Hallam, 2007). They are considered to be among the most common infectious diseases affecting all age groups, from infants to the elderly (Bojić-Milicević et al., 2005). UTIs are the most common infections seen in hospitalized patients and the second most common, after respiratory tract infections, seen in the general population (Fragoulis et al., 2007). Bacteria are by far the most frequent cause of UTIs, and aerobic Gram-negative bacilli predominate (Clarridge et al., 1987; Elgaml et al., 2013). An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility test of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections (Jorgensen and Ferraro, 2009). In almost all cases there is a need to start treatment before the final microbiological results are available. Area-specific monitoring studies aims to gain knowledge about the type of pathogens res-

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Table 1. Interpretation chart for antimicrobial susceptibility pattern according to the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

Antimicrobial agent	Symbols	Disc content (µg)	Inhibition zone diameter (mm)		
			R	I	S
Ampicillin	AM	10	≤11	12-13	≥14
Amoxacillin	AX	25	≤13	14-16	≥17
Cephradine	CE	30	≤14	15-17	≥18
Cefuroxime	CXM	30	≤14	15-17	≥18
Cefoperazone	CEP	75	≤15	16-20	≥21
Cefepime	FEP	30	≤14	15-17	≥18
Imipenem	IMP	10	≤13	14-15	≥16
Amikacin	AK	30	≤14	15-16	≥17
Gentamicin	CN	10	≤12	13-14	≥15
Ciprofloxacin	CIP	5	≤15	16-20	≥21
Levofloxacin	LEV	5	≤12	13-15	≥16

R: Resistant, I: intermediate, S: sensitive.

ponsible for certain infection and their resistance patterns which may help the clinician to choose the right empirical treatment (Hryniewicza et al., 2001).

On the other hand, typing techniques are useful for establishing clonal relationships between individual isolates in hospital settings which are important to recognize nosocomial transmission and guide infection control practice. One of the most commonly used typing techniques which allows a higher degree of taxonomic discrimination and is useful for epidemiological study is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (de Freitas and Barth, 2002; Sazakli et al., 2005).

The aim of the study was to obtain data on susceptibility patterns of major pathogens isolated from both community acquired and hospital UTIs in Mansoura University Hospitals, Egypt towards different classes of antimicrobial agents used in the treatment of UTIs and also to investigate their epidemiological relatedness using SDS-PAGE technique.

MATERIALS AND METHODS

Bacterial isolates

Twenty five isolates of *Pseudomonas*, twenty five isolates of *Escherichia coli* and twenty isolates of *Klebsiella* were isolated from Mansoura University Hospitals, Dakahlia governorate, Egypt. All the bacterial isolates were obtained from urine clinical specimens. The specimens were processed immediately using standard procedures and the bacterial isolates were identified according to Barrow and Feltham (1993) and Collee et al. (1996). Thereafter, the bacterial isolates were re-identified to the species level by sequencing of their *16s-rRNA* gene according to Elgaml et al. (2013).

Antimicrobial agents

Antimicrobial agents used with their respective interpretation charts are shown in Table 1.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the isolates was done by standard disk diffusion method as described in the National Committee for Clinical Laboratory Standards (NCCLS, 2000) guidelines. Briefly, from primary isolation medium one bacterial colony was taken by flamed loop, suspended in 5 ml broth and incubated overnight at 37°C with shaking. The bacterial density was adjusted to 10^6 CFU/ml. Petri dishes with Mueller-Hinton agar (4 mm depth) were used for performing the test. Streaking of the bacterial suspension with a clinical swab was done on the entire agar surface in three different directions by rotating the plate at 60° angles after each streaking. Afterwards, Petri dishes were allowed to dry for 15-20 min at room temperature. Then, by using a flamed forceps, standard commercial paper discs of selected antibiotics were inserted to the agar plates and gently pressed down to ensure contact. The plates were incubated in inverted position at 37°C overnight. After incubation, the diameters of inhibition zones were measured to the nearest mm using a Vernier caliber. The experiments were repeated at least three times and the isolates were reported as sensitive or resistant from the respective interpretation charts (Table 1).

SDS-PAGE

Whole cell lysates of test isolates were prepared for SDS-PAGE analysis as described by Nakamura et al. (2002). Briefly, from primary isolation medium one bacterial colony was picked, suspended in 5 ml LB broth and incubated overnight at 37°C with shaking. Subsequently, the broth culture was centrifuged at 15,000 rpm for 15 min at 4°C. The sediment was resuspended in 5 ml phosphate buffer solution (PBS, pH 7.2). One milliliter of the suspension was transferred into 1.5 ml microcentrifuge tubes and centrifuged at 15,000 rpm for 15 min at 4°C. The sediment was suspended in 10 µl of 10% SDS (AppliCem) and an equal volume of loading buffer [0.125 M Tris (hydroxymethyl) aminomethane (Tris, AppliCem), 4% SDS, 10% 2-mercaptoethanol (Merck), 0.2% bromophenol blue (AppliCem); pH 6.8] was added. After vigorous shaking by vortex, the prepared samples were boiled for 10 min at 100°C, centrifuged for 1 min (15,000 rpm at 20°C) and the supernatants were stored at -20°C until use.

The SDS-PAGE was carried out by using 12% (w/v) separating and 4% (w/v) stacking gels as described by Laemmli (1970). The

Table 2. Geographical distribution and clinical sources of the bacterial isolates.

Clinical isolates	Clinical isolates	Numbers of isolates	Geographical distribution	Clinical source
***Pseudomonas* (25)**	*Pseudomonas aurigonsae* (20)	Five isolates (number 1, 3, 12, 13 and 20)	(UNC)	Urine
		Eight isolates (number 2, 5, 14, 21, 22, 23, 24 and 25)	(MUH)	
		Seven isolates (number 7, 8, 9, 15, 16, 18 and 19)	(PUH)	
	Pseudomonas fluorescens (3)	Three isolates (number 6, 10 and 11)	(MUH)	
	Pseudomonas putida (2)	One isolate (number 4)	(MUH)	
		One isolate (number 17)	(PUH)	
***Escherichia* (25)**	*Escherichia coli* (25)	Two isolates (number 18 and 20)	(UNC)	
		Eleven isolates (number 4, 6, 8, 9, 10, 11, 12, 15, 16, 17 and 21)	(MUH)	
		Twelve isolates (number 1, 2, 3, 5, 7, 13, 14, 19, 22, 23, 24 and 25)	(PUH)	
***Klebsiella* (20)**	*Klebsiella pneumoniae* (18)	Sixteen isolates (number 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 15, 16, 17, 18, 19 and 20)	(UNC)	
		Two isolates (number 12 and 13)	(PUH)	
	Klebsiella oxytoca (2)	One isolate (number 6)	(UNC)	
		One isolate (number 14)	(PUH)	

(UNC) Urology and Nephrology Center, (MUH) Mansoura University Hospital, (PUH) Pediatric University Hospital

protein concentrations of the whole cell lysates were measured according to the method of Lowry et al. (1951). Five microliters of samples were electrophoresed on 12% acrylamide (Sigma) gel for 3 h at 30 mA using a small electrophoresis chamber (Thermo EC120 Mini Gel Vertical System, USA). In each gel a molecular weight mar-marker (Sigma) was included. The gels were stained in 0.25% Coomassie Brilliant Blue R250 (Sigma) in methanol: acetic acid: distilled water (5: 1: 5) for 90 min with gentle shaking. Then the gels were destained in methanol: acetic acid: distilled water (2:3:35) overnight and visualized with the GI-5000 visualization system (Spectronics Co., USA).

Similar SDS-PAGE patterns were considered as one pattern. Accordingly, the tested isolates were successfully classified into different SDS-PAGE patterns.

RESULTS AND DISCUSSION

Bacterial isolates

The overall species distribution is shown in Table 2.

Antimicrobial susceptibility testing

Pseudomonas isolates showed the highest antimicrobial resistance rate and was significantly resistant to most of the antimicrobials than other isolates. All *Pseudomonas* isolates (100%) were resistant to ampicillin, amoxicillin, cephradine, cefuroxime and cefoperazone, 96% were resistant to cefepime, 28% were resistant to imipenem, 48% were resistant to amikacin, 68% were resistant to gentamicin, 52% were resistant to both ciprofloxacin and levofloxacin (Table 3).

These results are in accordance with study in Iran conducted by Farajnia et al. (2009) showing that, *Pseudomonas* isolates possessing the highest antibiotic resistance rate was significantly resistant to most of the antimicrobials. Moreover these resistance profiles were higher than that reported in El-Astal (2004) study which showed 14.8% resistance for ceftriaxone, 95.4% for amoxicillin, 26.1% for gentamicin, 8.3% for amikacin and 17.8 % for ciprofloxacin. Also in Minia, Egypt, Gad et al. (2008) found that, *Pseudomonas* urinary tract infection isolates were 100% resistant to ampicillin and amoxicillin and were highly resistance to both quinolones and aminoglycosides antibiotics, where 95% of the isolates were resistant to azithromycin.

Regarding *Escherichia* isolates, 96% of the isolates were resistant to both ampicillin and amoxicillin, 92% were resistant to cephradine, 32% were resistant to cefuroxime, 20% were resistant to both cefoperazone and cefepime, 24% were resistant to gentamicin, 36% were resistant to both ciprofloxacin and levofloxacin. On the other hand, 100% of isolates were sensitive to both amikacin and imipenem (Table 4).

These results are in accordance with Zhao et al. (2009) who reported that, resistance rate of *E. coli* isolates to ampicillin was greater than 90% and all the strains were multidrug resistant. And by comparison of these results with that reported by Raka et al. (2004); they reported lower resistance to amoxicillin, ampicillin, ciprofloxacin and gentamicin than that obtained in the present study

Table 3. Antimicrobial sensitivity patterns of the isolated *Pseudomonas* species.

Isolate number	β-Lactams							Aminoglycosides		Quinolones		Number of antimicrobial agents isolates resistant to (N)
	AM	AX	CE	CXM	CEP	FEP	IMP	AK	CN	CIP	LEV	
1	R	R	R	R	R	R	S	S	S	R	R	**8**
2	R	R	R	R	R	R	S	S	R	R	R	**9**
3	R	R	R	R	R	R	S	R	R	R	R	**10**
4	R	R	R	R	R	R	S	S	R	S	S	**7**
5	R	R	R	R	R	R	S	R	R	S	S	**8**
6	R	R	R	R	R	R	R	S	R	R	R	**10**
7	R	R	R	R	R	R	S	S	S	R	R	**8**
8	R	R	R	R	R	R	S	S	S	S	S	**6**
9	R	R	R	R	R	R	R	R	R	S	S	**9**
10	R	R	R	R	R	R	R	R	R	S	S	**9**
11	R	R	R	R	R	R	S	R	R	S	S	**8**
12	R	R	R	R	R	R	S	S	S	S	S	**6**
13	R	R	R	R	R	R	S	S	R	R	R	**9**
14	R	R	R	R	R	R	S	R	R	R	R	**10**
15	R	R	R	R	R	R	R	S	S	R	R	**9**
16	R	R	R	R	R	R	S	R	R	R	R	**10**
17	R	R	R	R	R	R	S	R	R	R	R	**10**
18	R	R	R	R	R	R	S	R	R	R	R	**10**
19	R	R	R	R	R	R	R	R	R	S	S	**9**
20	R	R	R	R	R	R	S	S	S	S	S	**6**
21	R	R	R	R	R	R	R	R	R	S	S	**9**
22	R	R	R	R	R	R	R	R	R	S	S	**9**
23	R	R	R	R	R	R	S	S	R	R	R	**9**
24	R	R	R	R	R	R	S	S	S	R	R	**8**
25	R	R	R	R	R	S	S	S	S	S	S	**5**

AM: Ampicillin, AX: Amoxicillin, CE: Cephradine, CXM: Cefuroxime, CEP: Cefoperazone, FEP: Cefepime, IPM: Imipenem, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, LEV: Levofloxacin, S: sensitive, R: resistant.

Table 4. Antimicrobial sensitivity patterns of the isolated *Escherichia* species.

Isolate number	β-Lactams							Aminoglycosides		Quinolones		Number of antimicrobial agents isolates resistant to
	AM	AX	CE	CXM	CEP	FEP	IMP	AK	CN	CIP	LEV	
1	R	R	R	S	R	R	S	S	S	S	S	5
2	R	S	R	S	S	S	S	S	S	S	S	2
3	R	R	R	S	S	S	S	S	S	S	S	3
4	R	R	R	R	S	S	S	S	S	S	S	4
5	S	R	S	S	S	S	S	S	S	S	S	1
6	R	R	R	R	R	R	S	S	R	R	R	9
7	R	R	R	S	S	S	S	S	S	S	S	3
8	R	R	R	S	S	S	S	S	R	S	S	4
9	R	R	R	R	R	R	S	S	R	R	R	9
10	R	R	R	R	S	S	S	S	R	R	R	7
11	R	R	S	S	S	S	S	S	S	R	R	4
12	R	R	R	R	R	R	S	S	R	R	R	9
13	R	R	R	S	S	S	S	S	S	S	S	3
14	R	R	R	S	S	S	S	S	S	R	R	5
15	R	R	R	R	S	S	S	S	S	S	S	4

Table 4. Contd.

16	R	R	R	S	S	S	S	S	R	R	R	6
17	R	R	R	S	S	S	S	S	S	R	R	5
18	R	R	R	R	S	S	S	S	S	S	S	4
19	R	R	R	S	S	S	S	S	S	S	S	3
20	R	R	R	S	S	S	S	S	S	S	S	3
21	R	R	R	S	S	S	S	S	S	S	S	3
22	R	R	R	S	S	S	S	S	S	S	S	3
23	R	R	R	S	S	S	S	S	S	S	S	3
24	R	R	R	R	R	R	S	S	S	R	R	8
25	R	R	R	S	S	S	S	S	S	S	S	3

AM: Ampicillin, AX: Amoxicillin, CE: Cephradine, CXM: Cefuroxime, CEP: Cefoperazone, FEP: Cefepime, IPM: Imipenem, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, LEV: Levofloxacin, S: sensitive, R: resistant.

where 43% of isolates were resistant to amoxicillin, 41% to ampicillin, 1% to ciprofloxacin and 9.7% to gentamicin and higher resistance to amikacin than that obtained in the present study where 4.9% of isolates were resistant. Also, the results of the present study are higher than that recorded in a study in Iran conducted by Farajnia et al. (2009) showing the rates of resistance of *E. coli* isolates as the predominant cause of UTI, to a panel of antibiotics, including penicillins, cephalosporins, quinolones and aminoglycosides, which are routinely used to treat UTI infections. Moreover, these percentages of resistance of *E. coli* isolates are lower than that obtained by Akram et al. (2007) who reported different sensitivity patterns of some antimicrobial agents against *E. coli* isolates isolated from urinary tract infections in India. They reported that 65% of *E. coli* isolates were resistant to ceftazidime, 56% to cefotaxime, 55% to ceftriaxone, 64% to gentamicin, 73% to tobramicin, 51% to amikacin and 69% to ciprofloxacin and norfloxacin.

Regarding *Klebsiella* isolates, 100% of isolates were resistant to both ampicillin and amoxicillin, 90% were resistant to cephradine, 70% were resistant to cefuroxime, 60% were resistant to both cefoperazone and cefepime, 25% were resistant to amikacin, 40% were resistant to gentamicin, 20% were resistant to ciprofloxacin, 15% were resistant to levofloxacin. On the other hand, 100% of isolates were sensitive to imipenem (Table 5).

These results are higher than that reported by Farajnia et al. (2009) study, where 91.1% of *Klebsiella* spp. were resistant to ampicillin, 1.3% were resistant to amikacin, 19% were resistant to gentamycin and 1.3% were resistant to ciprofloxacin. In addition, the results are higher than the results of Barisic et al. (2003) who reported that 93.6% of *Klebsiella* isolates were resistant to amoxicillin, 32.7% to ampicillin, 28% to cefuroxime and 14% to norfloxacin. Moreover, by comparing these results with that reported by Raka et al. (2004), lower resistance to amoxicillin, ampicillin and ciprofloxacin was recorded, where 76% of isolates were resistant to amoxicillin, 49% were resistant to ampicillin, 8% were resistant to ciprofloxacin. In contrast, higher resistance to aminoglycosides was recorded where 54% of isolates were resistant to gentamicin and 51% were resistant to amikacin.

From the results of the sensitivity patterns of all isolates it was clear that there are many differences of antibiotic sensitivity test between different studies. These differences may be attributed to the fact that resistance rates vary from country to country (Gales et al., 2001).

Moreover, imipenem was the most efficient antimicrobial agents among all isolates, where all *Escherichia* and *Klebsiella* isolates (100%) and 72% of *Pseudomonas* isolates were sensitive to this antimicrobial agent. This increase in the resistance rate of *Pseudomonas* isolates to imipenem is due to its extensive use and this is in accordance with study conducted by Troillet et al. (1997). They reported parallel increase in resistance to imipenem with its use among Gram-negative bacilli and particularly *Pseudomonas*.

SDS-PAGE typing

SDS-PAGE is currently one of the most commonly used techniques for the characterization and analysis of proteins and it has been used as a taxonomic tool for identification of various bacterial species and yielding valuable information on the similarity and dissimilarity amongst bacterial cultures (Chung, 1987). The polyacrylamide gel electrophoresis (PAGE) of proteins analysis has been used widely in typing of many bacterial strains. Protein patterns offer considerable potential for typing bacterial strains of clinical interest, especially for species with other typing methods are not available (Holmes et al., 1991; Malik et al., 2003).

In the present study, protein profiles were very similar and characteristic among the isolates of each group of microorganisms and several isolates exhibited characteristic proteins that may be useful markers for epidemiological investigation.

SDS-PAGE of total cell protein extracts of 25 tested *Pseudomonas* isolates produced characteristic patterns containing about 32 discrete bands with molecular weights

Table 5. Antimicrobial sensitivity patterns of the isolated *Klebsiella* species.

Isolate number	β-Lactams							Aminoglycosides		Quinolones		Number of antimicrobial agents isolates resistant to
	AM	AX	CE	CXM	CEP	FEP	IMP	AK	CN	CIP	LEV	
1	R	R	R	R	R	R	S	R	R	R	R	10
2	R	R	R	R	S	S	S	S	S	S	S	4
3	R	R	R	R	R	R	S	R	R	S	S	8
4	R	R	R	R	R	R	S	R	R	S	S	8
5	R	R	R	R	R	R	S	S	S	S	S	6
6	R	R	R	R	R	R	S	R	R	S	S	8
7	R	R	R	S	S	S	S	S	S	S	S	3
8	R	R	R	R	R	R	S	S	S	S	S	6
9	R	R	R	R	R	R	S	S	R	R	R	9
10	R	R	R	R	R	R	S	S	R	S	S	7
11	R	R	R	S	S	S	S	S	S	S	S	3
12	R	R	R	S	S	S	S	S	S	S	S	3
13	R	R	R	S	S	S	S	S	S	S	S	3
14	R	R	S	S	S	S	S	S	S	S	S	2
15	R	R	R	R	R	R	S	S	S	S	S	6
16	R	R	R	R	R	R	S	S	R	R	S	8
17	R	R	R	R	R	R	S	S	S	S	S	6
18	R	R	S	S	S	S	S	S	S	S	S	2
19	R	R	R	R	S	S	S	S	S	S	S	4
20	R	R	R	R	R	R	S	R	R	R	R	10

AM: Ampicillin, AX: Amoxicillin, CE: Cephradine, CXM: Cefuroxime, CEP: Cefoperazone, FEP: Cefepime, IPM: Imipenem, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, LEV: Levofloxacin, S: sensitive, R: resistant.

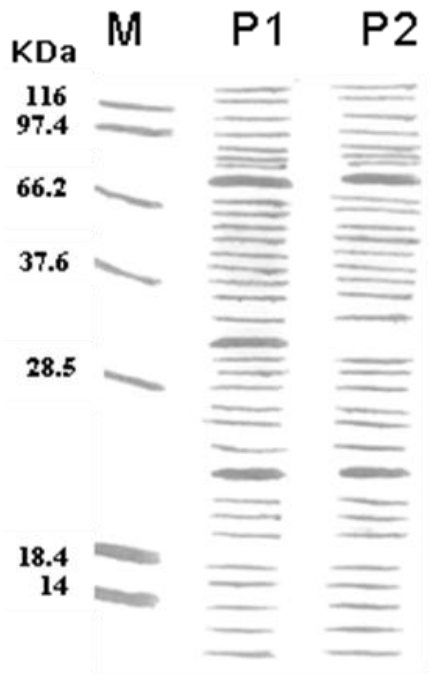

Figure 1. Schematic representation of different total cell protein patterns of *Pseudomonas* isolates. Lane M is molecular weight marker. Lanes P1 and P2 are patterns No. 1 and 2, respectively.

in the range from 14.4 to 116 KDa estimated by poly-acrylamide gel electrophoresis. The patterns among all tested isolates were nearly the same; however, few differences were observed. In the present study, two different total cell protein patterns were detected by SDS-PAGE (Figure 1). The first pattern was represented by 21 isolates (No. 1, 2, 3, 6, 7, 8, 9, 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25) and the second pattern was represented by 4 isolates (No. 4, 5, 11 and 13). The difference between the two patterns is that the second pattern lacks a band at about 33.05 KDa. The results figure out the sensitivity of SDS-PAGE as a powerful tool allowing a higher degree of taxonomic discrimination and for typing and subtyping of microorganisms even at the subspecies level, where out of 20 *P. aeruginosa* species identified by *16s-rRNA* sequencing 18 belonged to the first pattern (No. 1, 2, 3, 7, 8, 9, 12, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24 and 25) and 2 belonged to the second pattern (No. 5 and 13). Also out of 3 *P. fluorescens* species identified by *16s-rRNA* sequencing two belonged to the first pattern (No. 6 and 10) and one belonged to the second pattern (No. 11). Moreover, out of two *P. putida* species identified by *16s-rRNA* sequencing one belonged to the first pattern (No. 17) and was belonged to the second pattern (No. 4).

SDS-PAGE of total cell protein extracts of 25 tested *E. coli* isolates produced patterns containing about 30 discrete

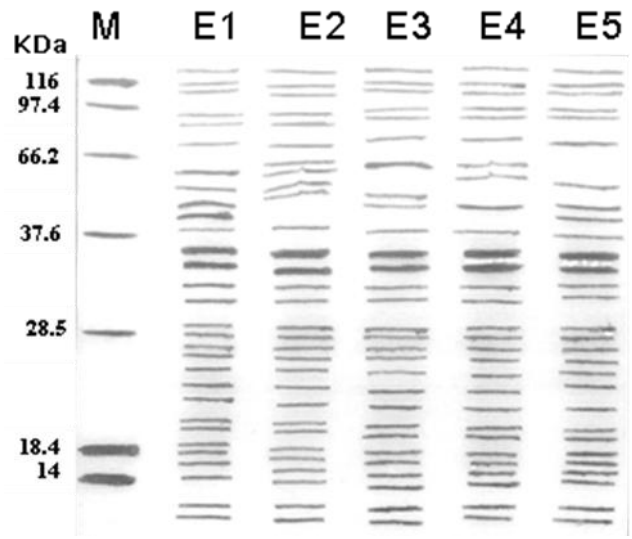

Figure 2. Schematic representation of different total cell protein patterns of *E. coli* isolates. Lane M is molecular weight marker. Lanes E1 to E5 are patterns No. 1, 2, 3, 4 and 5, respectively.

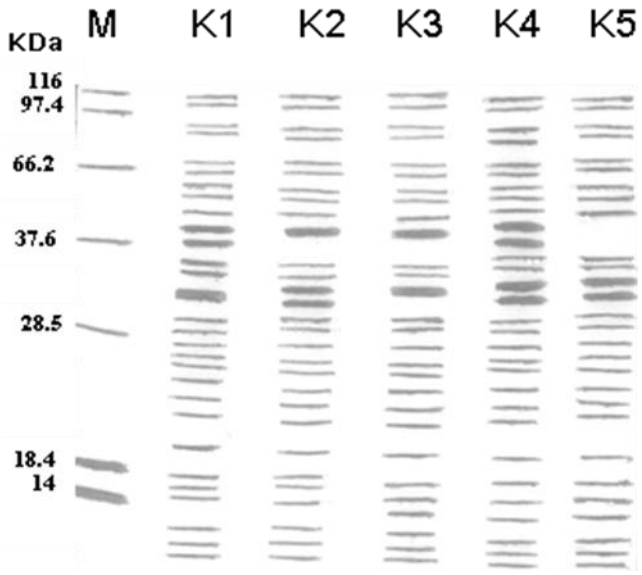

Figure 3. Schematic representation of different total cell protein patterns of *Klebsiella* isolates. Lane M is molecular weight marker. Lanes K1 to K5 are patterns No. 1, 2, 3, 4 and 5, respectively.

bands with molecular weights in the range fof 14.4 to 116 KDa estimated by polyacrylamide gel electrophoresis. The patterns among all tested isolates were nearly the same; however, there were few differences observed. In the present study, 5 different total cell protein patterns were detected by SDS-PAGE (Figure 2). The first pattern was represented by 10 isolates (No. 1, 5, 8, 9, 10, 14, 17, 18, 21 and 25), the second pattern was represented by 4 isolates (No. 2, 11, 19 and 24), the third pattern was represented by one isolate (No. 3), the fourth pattern was represented by 7 isolates (No. 4, 7, 12, 13, 16, 22 and 23) and the fifth pattern was represented by 3 isolates (No. 6, 15 and 20). From the results, it was clear that total cell protein patterns of *E. coli* isolates are very characteristic showing 2 adjacent characteristic heavy bands among all isolates at about 35 and 34.5 KDa. The main difference between the five total cell protein patterns were the bands between 66.2 and 37.6 KDa.

SDS-PAGE of total cell protein extracts of 20 tested *Klebsiella* isolates produced patterns containing about 30 discrete bands with molecular weights in the range of 14.4 to 116 KDa estimated by polyacrylamide gel electrophoresis. The patterns among all tested isolates were nearly the same; however, there were few differences observed. In the present study, 5 different total cell protein patterns were detected by SDS-PAGE (Figure 3). The first pattern was represented by 6 isolates (No. 1, 6, 7, 10, 15 and 16), the second pattern was represented by 3 isolates (No. 3, 9 and 18), the third pattern was represented by 3 isolates (No. 2, 4 and 19), the fourth pattern was represented by 3 isolates (No. 8, 11 and 17) and the fifth pattern was represented by 5 isolates (No. 5, 12, 13, 14 and 20). The main difference between the five total cell protein patterns were the bands at about 39 and 33.1 KDa. With *Pseudomonas* isolates, these results figure out the sensitivity of SDS-PAGE as a powerful tool allowing a higher degree of taxonomic discrimination and for typing and subtyping of microorganisms even at the subspecies level, where out of 18 *K. pneumonia* species identified by *16s-rRNA* sequencing 5 belonged to the first pattern (No. 1, 7, 10, 15 and 16), 3 belonged to the second pattern (No. 3, 9 and 18), 3 belonged to the third pattern (No. 2, 4 and 19), three belonged to the fourth pattern (No. 8, 11 and 17) and 4 belonged to the fifth pattern (No. 5, 12, 13 and 20). Also out of two *Klebsiella oxytoca* species identified by *16s-rRNA* sequencing, one was belonged to the first pattern (No. 6) and one belonged to the fifth pattern (No. 14).

Conclusion

This study shows:

1. The predominance of Gram-negative bacilli as the principal causative pathogens of UTIs.
2. The predominance of *E. coli* as the principal causative pathogen of UTIs, *P. aeruginosa* over other *Pseudomonas* species and *K. pneumoniae* over other *Klebsiella* species as an epidemiological marker.
3. Antimicrobial susceptibility testing showed that imipenem could be considered as the drug of choice for treatment infections caused by multi-resistant isolates of UTIs.
4. The sensitivity of SDS-PAGE as a powerful tool allowing a higher degree of taxonomic discrimination and for

typing and subtyping of microorganisms even at the subspecies level.

REFERENCES

Akram M, Shahid M, Khan AU (2007). Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann. Clin. Microbiol. Antimicrob.. 23:6-14.

Barisic Z, Babic-Erceg A, Borzic E, Zoranic V, Kaliterna V, Carev M (2003). Urinary tract infections in South Croatia: aetiology and antimicrobial resistance. Int. J. Antimicrob. Agents 2:61-64.

Barrow GI, Feltham RK (1993). Cowan and Steel's Manual for the identification of Medical Bacteria 3rd Ed., University press, Cambridge, U.K. 136-138.

Bojić-Milicević G, Mikov M, Dautović R (2005). Common errors in diagnosis and management of urinary tract infections--microbiological aspects. Med. Pregl. 58:380-387.

Chung MCM (1987). Polyacrylamide gel electrophoresis. In: Genes and proteins. A laboratory manual of selected techniques in molecular biology. Jeyaseelan K, Chung MCM, Konof, et, Bangkok. UNDP/WB/WHO. Special Programme Res. Training Trop. Dis. pp. 99-112.

Clarridge JE, Pezzlo MT, Vosti KL (1987). Cumitech 2A, Laboratory diagnosis of urinary tract infections. Coordinating ed., A. L. Weissfeld. American Society for Microbiology, Washington, D.C.

Collee JG, Miles RS, Watt B (1996). Tests for the identification of bacteria. In: Mackie and Mccartney. Practical Medical Microbiology, 14th Edn., I:413-424. Churchill Livingstone, N.Y, U.S.A.

de Freitas ALP, Barth AL (2002). Antibiotic resistance and molecular typing of *Pseudomonas aeruginosa*: focus on imipenem. Braz. J. Infect. Dis. 6:1-7.

El-Astal Z (2004). Bacterial pathogens and their antimicrobial susceptibility in GAZA STRIP, PALESTINE. Pak. J. Med. Sci. 20:365-370.

Elgaml A, Hassan R, Barwa R, Shokralla S, El-Naggar W (2013) Analysis of 16S ribosomal RNA gene segments for the diagnosis of Gram negative pathogenic bacteria isolated from urinary tract infections. Afr. J. Microbiol. Res. 7:2862-2869.

Farajnia S, Alikhani MY, Ghotaslou R, Naghili B, Nakhlband A (2009). Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. Int. J. Infect. Dis. 13:140-144.

Fragoulis KN, Vardakas KZ, Falagas ME (2007) Open access World Wide Web resources on urogenital infections. Nephrol. Dial. Transpl. 22:3046-3047.

Gad GF, El-Domany RA, Ashour HM (2008) Antimicrobial susceptibility profile of *Pseudomonas aeruginosa* isolates in Egypt. J. Urol. 180:176-181.

Gales AC, Jones RN, Turnidge J, Rennie T, Ramphal R (2001). Characterization of *Pseudomonas aeruginosa* isolates: occurrence rates, antimicrobial susceptibility patterns and molecular typing in the global SENTRY antimicrobial surveillance program 1997–1999. Clin. Infect. Dis. 32:46-55.

Holmes B, Costas M, Wood AC (1991). Typing of *Proteus mirabilis* from clinical sources by computerized analysis electrophoretic protein patterns. J. Appl. Bacteriol. 71:467-476.

Hryniewicza K, Szczypab K, Sulikowskab A, Jankowskia K, Betlejewskab K, Hryniewiczb W (2001). Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. J. Antimicrob. Chemother. 47:773-780.

Jorgensen JH, Ferraro MJ (2009) Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clin. Infect. Dis. 49:1749-1755.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nat. 227:680-685.

Lowry OH, Rosebrough J, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Malik A, Hasani SE, Shahid M, Khan HM, Ahmed AJ (2003). Nosocomial *Klebsiella* in neonates in a tertiary care hospital: Protein by SDS-PAGE and Klebocin typing as epidemiological markers. Indian J. Med. Microbiol. 21:82-86.

Naish W, Hallam M (2007). Urinary tract infection: diagnosis and management for nurses. Nurs. Stand. 21:50-57.

Nakamura A, Ota Y, Mizukami A, Ito T, Ngwai YB, Adachi Y (2002). Evaluation of aviguard, a commercial competitive exclusion product for efficacy and after effect on the antibody response of chickes of *Sallmonella*. Poult. Sci. 81:1653-1660.

National Committee for Clinical Laboratory Standards (2000). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically-Fifth Edition: Approved Standard M7-A2. NCCLS, Villanova, PA.

Raka L, Muliqi-Osmani G, Berisha L, Lumturije Begolli L, Omeragiq S, Parsons L, Salfinger M, Arbëresha Jaka A, Kurti A, Jakupi X (2004). Etiology and susceptibility of urinary tract isolates in Kosova. Int. J. Antimicrob. Agents 23:2-5.

Sazakli E, Leotsinidis M, Vantarakis A, Papapetropoulou M (2005). Comparative typing of *Pseudomonas* species isolated from the aquatic envrionment in Greece by SDS-PAGE and RAPD analysis. J. Appl. Microbiol. 99:1191-1203.

Troillet N, Samore HM, Carmeli Y (1997). Imipenem resistant *Pseudomonas aeruginosa*: risk factors and antibiotic susceptibility patterns. Clin. Infect. Dis. 25:1094-1098.

Zhao L, Chen X, Zhu X, Yang W, Dong L, Xu X, Gao S, Liu X (2009). Prevalence of virulence factors and antimicrobial resistance of Uropathogenic *E. coli* in Jiangsu Province (China). Urology 74:702-707.

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