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ISOLATION OF ANTIBIOTIC-RESISTANT BACTERIA FROM SEWAGE WATER IN MOSUL CITY

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ABSTRACT : Bacteria were isolated from sewage water of different areas of Mosul city to determine the spread of antibiotic resistance in environment. Membrane filter method was used where isolates were grown in MacKonkey, *Salmonella-Shigella*, Sodium azide, mannitol salt and *Pseudomonas* isolation agars. To diagnose microorganisms; Gram staining, colony morphology and color, motility test, utilization of sugars together with biochemical tests were employed. The isolates were identified as *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Pseudomonasaeruginosa* and *Enterococcus faecalis*. Antibiotic sensitivity testing was performed using trimethoprim, streptomycin, Rifampin, erythromycin, cephalothin, ciprofloxacin, carbenicillin, amoxicalv, amoxicillin and ampicillin. Isolates showed 50-100% resistance to antibiotics used where all were resistant to rifampin, amoxicalv and amoxicillin and *P. aeruginosa* was resistant to all. The results indicate high level of antibiotic resistance in sewage water in this city which may cause difficulty in treatment of infections when this multiresistance reaches human and animal pathogens.

Key words : Antibiotic-resistant bacteria, sewage water, motility test, infections, multiresistance.

INTRODUCTION

Microorganisms have developed antibiotic resistance to almost all currently available antibiotics via antibiotic resistance genes that are transmitted by extrachromosomal elements referred to as plasmids. This is manifested by (1) mutational changes on target site where an antibiotic acts, (2) reduction of drug availability either by a change in cell permeability to an antibiotic or efflux systems that pumps the antibiotic outside the bacterial cell, (3) synthesis of enzymes that modify or degrade the antibiotic, and (4) bypassing that target where an antibiotic acts through alternative pathway (D•idiæ *et al*, 2008; Munita and Arias, 2016).

Bacteria present in the environment act as reservoir for antibiotic resistance genes. In the study of Al-Berfkani *et al* (2014) on water samples collected from water works in Duhok city, Iraq;*Staphylococcus aureus*, *Micrococcus varians* and *Aeromonas hydrophila* were resistant to penicillin, lincomycins, amoxicillin and co-trimoxazole while the study of Sood *et al* (2015) on antibiotic resistant pathogens isolated from environmental water samples out from hospital in Ahmedabad, India showed that *Escherichia coli* isolates had 33.34%, 16.67% and 8.34% resistance to tetracycline, co-trimoxazole and ciprofloxacin, respectively.

The aim of this study is to identify the antibiotic resistance profiles in bacteria isolated from sewage system in Mosul city, Iraq.

MATERIALS AND METHODS

Collection of samples

250 ml of sewage water samples were collected from five areas of right sector in Mosul city. Membrane filter method was used according to Collins *et al* (2004). Briefly water samples were filtered through 0.45 µm and the membrane was placed on a selective medium for growth of isolates. Media used are tetrathionate broth, *Salmonella-Shigella* agar, MacConkey agar, sodium azide agar, mannitol salt agar, *Pseudomonas* isolation agar.

Identification of bacterial isolates

To identify bacteria, Gram stain, motility test, colony morphology and color were studied in addition to biochemical tests according to Winn *et al* (2006). These tests include oxidase, catalase, IMViC, gelatin hydrolysis and utilization of carbohydrates.

Antibiotic sensitivity testing

This test was performed using Kirby-Bauer method

in which Mueller-Hinton agar was used to inoculate bacteria and according to guidelines of National Committee of Clinical Laboratory Standards (NCCLS, 2002). The following antibiotics were used: Trimethoprim (TMP), streptomycin (S), Rifampin (RA), erythromycin (E), cephalothin (Kf), ciprofloxacin (Cip), carbenicillin (cb), amoxicalv (Ame), amoxicillin (Amox) and ampicillin (Amp). These antibiotics were purchased from Bioanalyse, Turkey.

Plasmid purification and electrophoresis

Plasmid DNA was purified according to Brinboim and Doly (1979) with slight modifications. Bacteria were grown overnight and centrifuged at 8000 rpm for 10 min. Pellet was suspended in a solution of lysozyme and Tris-HCl and placed on ice for 30 min. A solution of 2 M NaOH and 10% SDS was added and followed by sodium acetate at PH 8.0. The mixture was placed on ice for 60 min. Centrifugation was performed at 5 min and the precipitate was discarded. 1 ml of 95% cold ethanol was added to the supernatant. The plasmid DNA was collected by centrifugation for 10 min and the precipitate was suspended in a solution of 1 M sodium acetate and Tris-HCl at pH 8.0. Plasmid DNA was precipitated again with cold ethanol and the tube was placed in -20°C for 30 min. To remove ethanol, centrifugation was carried out and the DNA was dried in air with vacuum. The precipitate was dissolved in 20 µl of Tris-EDTA buffer. The DNA concentration and its purity were measured in Nanodrop 2000 spectrophotometer (ThermoFisher, USA).

After the extraction of DNA from the bacterial cells,agarose gel electrophoresis was performed on the plasmid DNA at 80 V. Briefly 1% agarose gel was prepared. 5µl of the loading dye was mixed with the plasmid DNAsamples and it was transferred into the wells. The bandswerevisualized under UV transilluminator for the bandsof plasmids.1500 base pair DNA marker was used.

RESULTS AND DISCUSSION

Table 1 shows the bacterial isolates with their identification tests while Table 2 shows their antibiotic sensitivity patterns. *Pseudomonas aeruginosa* was resistant to all antibiotics tested. *Salmonella typhi* had the lowest resistance of 50% to the antibiotics used. The rest were 70-80% resistant to the antibiotics used in testing. All the isolates were resistant to rifampin, amoxicalv and amoxicillin and all except *S. typhi* are also resistant to ampicillin and trimethoprim. Aziz *et al* (2014) also isolated *E. coli*, *Klebsiella pneumoniae*, *S. typhi* and *P. aeruginosa* among other genera from sewage water of hospital in Erbil city of Iraq. For *E. coli* of their research

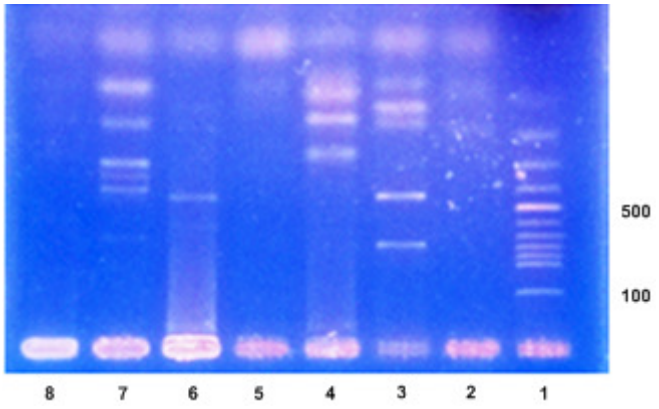

Fig. 1 : Plasmid profile of the isolates. (1) 1500 bp ladder, (2) *S. typhi* (3) *P. aeruginosa*, (4) *E. faecalis*, (5) *Klebsiella* spp., (6) *Proteus* spp., (7) *S.aureus* and (8) *Enterobacter* spp.

80% of resistance to ampicillin, 77% resistance for amoxiclav and 22-60% resistance to cefotaxime, meropenem, imipenem, azetreonam, ceftazidime, cefepime and pipercillin/tazobactam. 41% of the isolated *E. coli* had resistance to more than four antibiotics.

Microbes resistant to commonly used antibiotics are found now in environment due to the widespread use of antibiotics in treating infections of human and animals in addition to their presence in both human and animal excreta. The use of antimicrobials had led to a selective pressure causing the appearance of pathogenic microorganisms that acquired antibiotic resistance genes (Kummerer, 2004; Raj, 2012).

The study of Akhter *et al* (2014) on coliform bacteria isolated from Gometi river receiving sewage water from domestic, hospitals and small industries showed multiresistant isolates of *E. coli* and *Enterobacter* spp. Resistance levels of *E. coli* was higher than *Enterobacter* spp. Regarding amoxicillin, erythromycin, ciprofloxacin and tetracyclin. Al-Zyadi and Mallah (2008) had isolated *P. aeruginosa* from soil in Muthana city, Iraq and 88.8% of the isolates were resistant to erythromycin and rifampin and 37% were resistant to streptomycin but all sensitive to ciprofloxacin.

Howard *et al* (2001), Pirko *et al* (2017) and Tektook (2018) studied the factors associated with antibiotic resistance and found that: (1) there is a correlation between trimethoprim administration and ampicillin resistance as linked-resistance pattern which is transmitted via plasmids (2) coliform species other than *E. coli* are associated with higher resistance pattern in young and elderly males (3) antibiotic resistance is associated with poverty due to higher incidence of diseases.

Fig. 1 shows electrophoresis of plasmid content for the isolates. Multiple bands were detected in *P. aeruginosa*, *E. faecalis* and *S. aureus*, which may suggest

Table 1 : Identification test of the bacterial isolates.

Microorganisms	Biochemical tests								Carbohydrates utilization				
	GS	Cat	Oxi	I	VP	MR	Cit	Gel	Glu	Sor	Gal	Lac	Man
E. coli	-	+-	+	-	+	-	-	+	+	+	+	-	
Klebsiella spp.	-	+-	-	+	-	+	-	+	+	+	+	-	
Enterobacter spp.	-	+-	-	+	-	+	-	+	-	+	+	-	
Proteus spp.	-	+-	-	-	+	+	+	+	-	+	-	-	
S. typhi	-	+-	-	-	+	-	-	+	+	+	-	-	
P. aeruginosa	-	++	-	-	-	+	-	+	-	+	-	-	
S. aureus	+	+-	-	+	+	+	+	+	-	+	+	+	
E. faecalis	+	--	v	+	v	v	+	+	-	+	v	-	

GS: Gram stain, Cat: catalase test, Oxi: oxidase test, I: indole test, VP: Voges-Prauskaeur test, MR: methyl red, Cit: citrate test, Gel: gelation hydrolysis, Glu: glucose, Sor: sorbitol, Galactose, Lac: lactose, Man: mannitol, v: variable.

Table 2 : Antibiotic sensitivity testing of the bacterial isolates.

Microorganisms	Antibiotics discs used in potencices (µg/disc)									
	TMP (30)	S(25)	RA(5)	E(10)	Kf(30)	Cip(10)	cb(25)	Ame(30)	Amox(20)	Amp (25)
E. coli	R	MS	R	R	R	S	R	R	R	R
Klebsiella spp.	R	S	R	R	R	S	R	R	R	R
Enterobacter spp.	R	R	R	R	R	S	R	R	R	R
Proteus spp.	R	R	R	R	R	S	S	R	R	R
S. typhi	MS	R	R	MS	S	S	R	R	R	MS
P. aeruginosa	R	R	R	R	R	R	R	R	R	R
S. aureus	R	MS	R	S	R	S	R	R	R	R
E. faecalis	R	R	R	R	R	S	R	R	R	R

S: sensitive, MS: intermediate sensitive and R: resistant. Inhibition zone diameterswere determined according to NCLLS (2002).

different types of plasmids are present. Antibiotic resistance are carried and transferred through plasmids among microbes. Al-Jumaily *et al* (2010) studied the plasmid content of water isolated from different areas of Iraq including Mosul city and found that *E.coli* isolates were resistant to ampicillin, amoxicillin, erythromycin, tetracycline, chloramphenicol, nalidixic acid, cefoxitin, metronidazole, ofloxacin and ceftizoxime. Two plasmids bands were detected in DNA electrophoresis, one was a megaplasmid. The study suggested that a similarity present in antibiotic resistance profile in bacteria isolated from hospitals and close areas where water samples are collected. Raj (2012) studied the transfer of resistance genes in bacteria isolated from sewage in Ghaziabad city of India. All multi-drug resistant *Enterobacter* spp. were resistant to penicillin, streptomycin, erythromycin, amikacin, neomycin but variable for other antibiotics. A single plasmid of 54.4 Kb was detected.

CONCLUSION

There is a significant increase of antibiotic resistance genes among environmental isolates which may cause a serious issue when these genes are transmitted to pathogenic organisms affecting human and animal. Sewage water has become a pool for dissemination of antibiotic resistance in the community and measure should be taken to solve this problem.

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