academicJournals

Vol. 9(19), pp. 1312-1321, 13 May, 2015
DOI: 10.5897/AJMR2015.7486
Article Number: DB9D1F553248
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Identification, antibiotic resistance and distribution of different classes of integrons among *proteus* species isolated from different sources in Dakahleia and Damietta Egyptian Governorates

Mohamed Adel El-Sokkary[1], Mohamed Mohamed Adel El-Sokkary[1]*, Rasha Aabed[2] and Rasha Barwa[1]

[1]Department of Microbiology and Immunology, Mansoura University, Mansoura, Egypt.
[2]General Governmental Hospital of Damietta Egypt.

Received 19 March, 2015; Accepted 7 May, 2015

The aim of the present investigation is to trace the epidemiological relatedness between different isolates of *proteus* sp. obtained from Dakahleia governorate as compared with similar sources from the adjacent Damietta governorate in Egypt. A total of 500 bacterial isolates were collected from human and animal sources including urine samples, sputum, wound, throat and ear swabs, endotracheal aspirate, breast swab, bile aspirate, blood samples, human and animal stools. From a total of 70 *Proteus* isolates, 62 were identified as *Proteus mirabilis* while eight as *Proteus vulgaris*. All *Proteus mirabilis* isolates were screened for susceptibility to ten different antimicrobials and were classified into 29 different patterns. Two resistant strains were found to belong to pattern No. 10 (isolates No. 11 and 19). A second pair of isolates namely No. 27&31 was classified into pattern No. 18. A third pair of (isolates No 30 and 16) was found to have great similarity to pattern No. 18 with a minor difference in resistance against one or two antibiotics. For a molecular characterization of the resistance determinants, 15 of the highly resistant *Proteus mirabilis* isolates were screened for the presence of different classes of integrons. Class 1 was the dominantly detected type that confers resistance to trimethoprim and aminoglycosides. One isolate was found to contain class 2. Similar integron components *(dfrA15*) could be proved among strains of different human sources (No. 11 & 19). Another similar type of intergon components *(dfrA17) -(aadA5)* was found in human isolates 31, 27, 30 & 16. In conclusion, three pairs of the studied isolates have been turned out to be epidemiologically related with the possibility of hospital infection and of strain transfer from one governorate to the other.

Key words: *Proteus*, integrons, resistance pattern, swarming.

INTRODUCTION

Bacteria of the genus *Proteus* are part of the normal flora of the intestinal tract of humans and animals and are widespread in the environment. In particular, *Proteus mirabilis* accounts for approximately 3% of nosocomial infections in the United States mainly causing cystitis, pyelonephritis, and prostatitis. In Egypt, *P. mirabilis* constitutes the third most commonly isolated pathogen (after *Escherichia coli* and *Klebsiella pneumoniae*) of urinary tract infections. They are mostly ascending infections, more common among patients with anatomical

or physiological malformations of the urinary tract, as well as among catheterized patients or due to medical care mistakes (Chen et al., 2012). Prevalence of colonization with resistant microorganisms within a hospital can occur through bacterial cross-transmission or contamination originating from an environmental source (Lipsitch and Samore, 2002). In addition, microorganisms can acquire resistance determinants through horizontal gene transfer. Studies on the epidemiology of *proteus* species are of great importance in developing countries. This genus constitutes a great importance due to the wide diversity of infections caused by its member namely *P. mirabilis*. *Proteus* sp. has been proved to be one of the important causative of hospital infection in developing countries (Wasfi et al., 2012). However, comparative studies of *proteus* sp. between hospitals or adjacent governorates are not well studied in Egypt. It is also of great importance to characterize the resistance pattern of *proteus* strains from different sources of the specified regions for future antibiotic treatment policy.

The aim of the present investigation is to trace any relatedness or discriminatory characteristics between *proteus* isolates of the same regional source of Dakahleia governorate and the other adjacent Damietta governorate. Discrimination will be on the basis of antimicrobial resistance pattern, class and molecular structure of integrons as a powerful indicator parameter for discrimination Basis.

METHODOLOGY

Bacterial isolates

A total of 500 bacterial isolates were obtained from Dakahleia and Damietta, governorates in Egypt.Bacterial samples were collected from Dakahleia governorates including: Mansoura University Hospital (MUH), Mansoura Emergency Hospital (MEH), Urology and Nephrology Center, Mansoura University (UNC), Specialized Medical Hospital, Mansoura University (SMH), Pediatric University Hospital (PUH), Automated Slaughter house in Mansoura (MAS) and Private Laboratories Specimens. Damietta isolates were obtained from National Cancer Institute (DNCI), Damietta General Hospital (DGH), Damietta Specialist Hospital (DSH), Damietta Central Joint Laboratory (DCJL), Farms from Damietta. Samples were collected from various sources including (70) urine samples, (30) sputum, (120) wound and (13) throat swabs, (15) ear swabs, (20) endotracheal aspirate, (5) breast swab, (2) bile aspirate, (25) blood samples, (100) human and (100) animal stools Protocol of such studies was approved by Institute IRB/EC before registration of the point as a Master thesis.

Phenotypic identification of *proteus* isolates

Identification of different *proteus* isolates was carried out by the usual phenotypic methods including cultural characteristics with swarming appearance. The bacilli are pleomorphic Gram-negative bacilli. Biochemical reactions including indole test and other biochemical reactions related to different *proteus* sp. were also verified (Crichton, 1996).

Antimicrobial susceptibility testing

All isolates were twice screened for susceptibility to ten antimicrobial discs namely; ampicillin (AMP, 10 µg), ampicillin/sulbactam (SAM, 10/10 µg), cefepime (FEP, 30 µg), cefoperazone (CFP, 75 µg), imipenem (IPM, 10 µg), cefoperazone and sulbactam SCF, 75 µg/30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), gentamicin (CN, 10 µg), tobramycin (TOP, 10 µg) using the standard disc All discs were supplied by Oxoid USA. The arithmetic mean to the nearest 0.5 mm was calculated for each antibiotic, and the results were categorized for each antibiotic according Clinical Laboratory Standards Institute Recommendation (CLSI, 2010).

Identification of different classes of integron for 15 resistant strains by Multiple PCR amplification

Identification of different classes of integron was carried out by a multiplex polymerase chain reaction (PCR). Amplification of genes representing the constant region of Class 1, 2 and 3 integrons was achieved by using the primer sets listed in Table 1. The template DNA for PCR was prepared as described by Zhang et al. (2004).

Characterization of the variable region of class 1 and class 2 integrons

Amplification of the variable region of class 1 and 2 integrons was performed using four primers 5-CS/3-CS and Ti-F/Ti-B, respectively, as described previously (Zhang et al., 2004). The reaction mixture was prepared as described previously. PCR reactions began with 10 minutes of primary denaturation at 94°C followed by 40 cycles of 94°C for 30 s, annealing temp, 30 s and 72°C for 30 s. Primers 5-CS/3-CS were annealed at 49°C while Ti-F/Ti-B reactions were annealed at 52°C.

Determination of the gene sequence of different classes of integron

Amplified gene fragments were purified using the PCR Purification Kit (MEGA quick-spin fragment DNA purification INTRON biotechnology, Sangdaewon-Dong, Korea) for Sequencing. Purified PCR products were used as a template in sequencing reactions carried out with the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Bio-systems, Foster City. USA). The reaction mixtures were analyzed on an ABI 3730 DNA analyzer (Applied Bio-systems, Foster City, USA). Amplicons were sequenced on both strands and predicted peptide sequences analyzed by the online BLAST of the NCBI website software (http://www.ncbi.nlm.nih.gov/BLAST/). Nucleotide sequences of the antibiotic resistance genes were deposited in Gene Bank under definite accession numbers (Table 1).

*Corresponding author. E-mail: m_elsokkary2022@yahoo.com.

Table 1. Primers used for amplification of class 1, 2 and three integrase genes and their variable regions

Primer	Sequence	Size of amplicon (bp)	Targets	GenBank number	References
intM1-U IntM1-D	5'-ACGAGCGCAAGGTTTCGGT-3' 5'-GAAAGGTCTGGTCATACATG-3'	565	*intI1*	AF550415	Jianyu et al., 2005
IntM2-U IntM2-D	5'-GTGCAACGCATTTTGCAGG-3' 5'-CAACGGAGTCATGCAGATG-3'	403	*intI2*	AP002527	Jianyu et al., 2005
IntM3-U IntM3-D	5'-CATTTGTGTTGTGGACGGC-3' 5'-GACAGATACGTGTTTGGCAA-3'	717	*IntI3*	AY219651	Jianyu et al., 2005
5'-CS 3'-CS	5'-GGCATACAAGCAGCAAGC-3' 5'-AAGCAGACTTGACCTGAT-3'	Variable	Gene cassette(s) of class 1 integron	U12338	Zhang et al., 2004
Ti-F Ti-B	5'- ACCTTTTTGTCGCATATCCGTG -3' 5'- CTAACGCTTGAGTTAAGCC -3'	Variable	Gene cassette(s) of class 2 integron	AJ289189	Jianyu et al., 2005

RESULTS

Phenotypic Identification of *Proteus* species

According to cultural characteristics and biochemical reactions, out of a total of 500 collected isolates of bacteria, seventy solates were identified as *Proteus* species, where 31 isolates were from Dakahleia and 39 isolates were isolated from Damietta. Subsequent identification revealed that 62 isolates were identified as *Proteus mirabilis* (Tables 2 and 3), and eight isolates were identified as *Proteus vulgaris* (Tables 2 and 3).

Antimicrobial susceptibility test

Regarding *P. mirabilis*, and according to Tables 2 and 3, twenty-nine different antibiotic susceptibility patterns were detected among the tested strains. Thirty-seven isolates (59.7%) were resistant to ampicillin, 15 isolates (24%) were resistant to gentamicin, cefoperazone, and ampicillin /sulbactam combination, 14 isolates (22.5%) were resistant to tobramycin and 7 isolates were resistant to ciprofloxacin (11.2%).

In contrast, all isolates (100%) were sensitive to cefoperazone /sulbactam combination. 58 isolates (93.5%) were sensitive to levofloxacin while 57 isolates (92%) were sensitive to imipenem, and 56 isolates (90.3%) were sensitive to cefepime.

Regarding *P. vulgaris*, five different antibiotic susceptibility patterns were detected among the tested isolates (Tables 2 and 3).The majority of the isolates were sensitive to most antimicrobial agents used in this study (Figure 1).

Identification and characterization of gene cassette of class 1, 2 and 3 integrons

Fifteen (15) resistant integron positive *P. mirabilis* were subjected to further investigation of their antibiotic resistance genes present in different classes of integron (Table 4). Only 6 isolates of *P. mirabilis* were found to carry detectable Class 1-related integrons, showing the existence of amplicons of 1665 bp in four, 1496 bp in one and 726-737 bp in three of the class 1 integron positive isolates. Class 2 was identified in only one isolate is having 857 bp amplicon. Class 3 integron could not be detected from any of the integrons studied isolates.

Nucleotide sequences of the antibiotic resistance genes were deposited in Gene Bank under accession numbers: KM386399, KM386400, KM386401, KM386402, KM386403, KM386404, KM386405, KM386406 and KM386407 (Table 4). Table 5 summarizes the main differences between the selected resistant strains including resistance pattern and integron characterization.

DISCUSSION

The aim of the present work is to study the

Table 2. Dakahleia isolates, their origin, sample source, isolation center, and antimicrobial resistance.

Species	Strain No.	Origin	Isolation center	Sample source	Sex	Antibiotyping profiles										Pattern No.
						AMP	CEP	FEP	IPM	SAM	SCF	CN	TOB	CIP	LEV	
P. mirabilis	1	Human	MUH	Wound	F	I	S	S	S	S	S	S	S	S	S	1
	2	Human	MUH	Wound	M	R	I	S	S	S	S	S	S	I	S	2
	3	Human	PUH	Blood	F	R	S	S	S	S	S	S	S	I	S	3
	4	Human	SMH	Ear swab	F	R	R	S	S	R	S	I	I	R	S	4
	5	Human	MEH	Wound	F	R	R	S	S	I	S	R	R	R	I	5
	11	Human	GEC	Endo tracheal aspirate	F	R	R	S	S	S	S	R	R	S	S	10
	12	Human	MCH	Oral swab	F	R	R	S	S	S	S	R	R	S	S	10
	13	Human	MEH	Urine	M	R	I	S	S	S	S	R	S	S	S	11
	14	Human	MCH	Sputum	F	S	S	S	S	S	S	S	S	S	S	12
	15	Human	MEH	Endotracheal tube	M	R	S	S	S	S	S	S	S	S	S	8
	19	Human	MUH	Wound	M	R	R	S	S	S	S	R	R	S	S	10
	20	Human	MEH	Wound	F	S	S	S	S	S	S	S	S	S	S	12
	21	Human	UNC	Urine	M	R	S	R	S	S	S	S	R	R	S	14
	22	Human	MUH	Wound	M	R	I	S	S	S	S	S	S	S	S	15
	24	Human	MUH	Breast swab	F	R	S	S	S	S	S	S	S	S	S	8
	25	Human	PUH	Blood	M	R	S	S	S	I	S	S	S	S	S	16
	27	Human	GEC	Bile aspirate	M	R	R	S	S	R	S	R	R	I	R	18
	29	Human	MEH	Wound	F	R	R	S	S	I	S	R	R	S	S	19
	36	Human	MCH	Sputum	F	I	S	S	S	S	S	S	S	S	S	1
	37	Human	MUH	Oral swab	F	S	S	S	S	S	S	S	S	S	S	12
	53	Human	Private clinic	Stool	F	R	R	S	S	S	S	I	I	I	S	27
	54	Human	Private clinic	Stool	F	I	S	S	I	S	S	S	S	S	S	23
	55	Human	Private clinic	Stool	M	S	S	S	S	S	S	S	S	S	S	12
	56	Human	Private clinic	Stool	F	R	I	I	S	S	S	S	S	I	S	28
	57	Human	Private clinic	Stool	F	S	S	S	S	S	S	S	S	S	S	12
	58	Human	Private clinic	Stool	M	S	S	S	S	S	S	S	S	S	S	12
	62	Animal	MAS	Rectal swab	F	S	S	S	S	S	S	S	S	S	S	12
	64	Animal	MAS	Rectal swab	F	S	S	S	S	S	S	S	S	S	S	12
P. vulgaris	52	Human	Private clinic	Stool	F	S	S	S	S	S	S	S	S	S	S	12
	63	Animal	MAS	Rectal swab	F	I	S	S	S	S	S	S	I	S	S	32
	65	Animal	MAS	Rectal swab	F	R	I	I	S	R	S	I	I	I	S	33

R: Resistant, I : Intermediate, S: Sensitive, AMP : ampicillin (10µg), CEP : cefoperazone (30µg), FEP : cefepime (30µg) , IPM : imipenem (10µg), SAM : ampicillin – sulbactam, SCF : cefoperazone (75µg) – sulbactam (30µg), CN : gentamicin (30µg), TOB : tobramycin (10µg), CIP : ciprofloxacin (5µg), LEV : levofloxacin (5µg). F: female, M : male MUH: Mansoura University Hospital, Mansoura University, MEH : Mansoura emergency hospital, UNC : urology and Nephrology Center, Mansoura University, SMH: specialized medical hospital , Mansoura University, PUH: pediatric university hospital, Mansoura University, GEC : gastroenterology surgical center, Mansoura University, MCH: Mansoura Chest Hospital. MAS: Mansoura automated slaughterhouse.

Table 3. Damietta isolates, their origin, sample source, isolation center, and antimicrobial resistance

Species	Strain No.	Origin	Isolation center	Sample source	Sex	Antibiotyping profiles										Pattern No.
						AMP	CEP	FEP	IPM	SAM	SCF	CN	TOB	CIP	LEV	
P. mirabilis	6	Human	GGHD	Ear swab	M	R	R	R	S	S	S	R	R	S	S	6
	7	Human	GGHD	Wound	M	R	R	S	S	R	S	S	R	S	S	7
	8	Human	DSH	Wound	M	R	S	S	S	S	S	S	S	S	S	8
	9	Human	DSH	Wound	M	R	R	S	S	R	S	S	R	S	S	7
	10	Human	DSH	Wound	F	R	R	R	R	R	S	S	S	S	S	9
	16	Human	GGHD	Urine	F	R	I	S	S	R	S	R	R	I	S	13
	17	Human	DSH	Urine	F	R	S	S	S	S	S	S	S	S	S	8
	18	Human	DSH	Urine	M	R	S	S	S	S	S	S	S	S	S	8
	23	Human	DSH	Wound	M	R	S	S	S	S	S	S	S	S	S	8
	26	Human	GGHD	wound	M	S	S	S	S	S	S	S	I	S	S	17
	30	Human	DSH	Urine	F	R	S	S	S	R	S	R	I	R	I	20
	31	Human	DCJL	Urine	F	R	R	S	S	R	S	R	R	I	R	18
	32	Human	DSH	Urine	M	R	S	S	S	I	S	I	I	S	S	21
	33	Human	DSH	Urine	M	R	S	S	S	S	S	S	S	S	S	8
	34	Human	DSH	Wound	F	R	S	S	S	S	S	S	S	S	S	8
	35	Human	DSH	Urine	M	R	S	S	S	S	S	S	S	S	S	8
	38	Human	DSH	Wound	F	S	S	S	S	S	S	S	S	S	S	12
	39	Human	DSH	Wound	M	S	S	S	S	S	S	S	S	S	S	12
	40	Human	DSH	Wound	F	I	S	S	S	S	S	S	S	S	S	1
	41	Human	GGHD	Stool	M	I	S	S	S	S	S	S	S	S	S	1
	42	Human	GGHD	Stool	F	S	S	S	S	S	S	S	S	S	S	12
	43	Human	Private clinic	Stool	F	R	R	R	R	R	S	R	R	R	R	22
	44	Human	Private clinic	Stool	F	I	S	S	I	S	S	S	S	S	S	23
	46	Human	GGHD	Stool	M	R	I	S	S	S	S	S	S	S	S	15
	47	Human	GGHD	Stool	F	I	I	S	S	S	S	S	S	S	S	24
	49	Human	Private clinic	Stool	M	S	S	S	S	S	S	S	S	S	S	12
	50	Human	GGHD	Stool	M	S	S	S	S	S	S	S	S	S	S	12
	51	Human	Private clinic	Stool	M	R	I	S	S	S	S	R	I	S	S	25
	60	Animal	Private farm	Rectal swab	NI	S	S	S	S	S	S	S	S	S	S	12
	61	Animal	Private farm	Rectal swab	NI	S	S	S	S	S	S	S	S	S	S	12
	66	Animal	Private farm	Rectal swab	F	R	R	I	I	I	S	R	S	R	S	26
	67	Animal	Private farm	Rectal swab	F	R	I	S	S	S	S	S	S	S	S	15
	69	Animal	Private farm	Rectal swab	F	S	S	S	S	S	S	S	S	S	S	12
	70	Animal	Private farm	Rectal swab	F	R	S	S	S	I	S	R	R	R	S	29
P. vulgaris	28	Human	DNCI	Sputum	M	R	I	R	S	I	S	R	S	S	R	30

Table 3. Contd

45	Human	GGHD	Stool	F	S	S	S	S	S	S	S	S	S	S	12
48	Human	Private clinic	Stool	F	R	I	S	I	S	S	S	S	S	S	31
59	Animal	Private farm	Rectal swab	NI	S	S	S	S	S	S	S	S	S	S	12
68	Animal	Private farm	Rectal swab	F	S	S	S	S	S	S	S	S	S	S	12

R: Resistant, I : Intermediate, S: Sensitive, AMP : ampicillin (10 µg), CEP : cefoperazone (30 µg), FEP : cefepime (30 µg) , IPM : imipenem (10 µg), SAM : ampicillin – sulbactam, SCF : cefoperazone (75 µg) – sulbactam (30 µg), CN : gentamicin (30µg), TOP : tobramycin (10 µg), CIP : ciprofloxacin (5 µg), LEV : levofloxacin (5 µg). F: female, M: male, NI: non-identified, DNCI: Damietta national cancer institute, GGHD: general governmental hospital of Damietta, DSH: Damietta specialist hospital, DCJL: Damietta central joint laboratory.

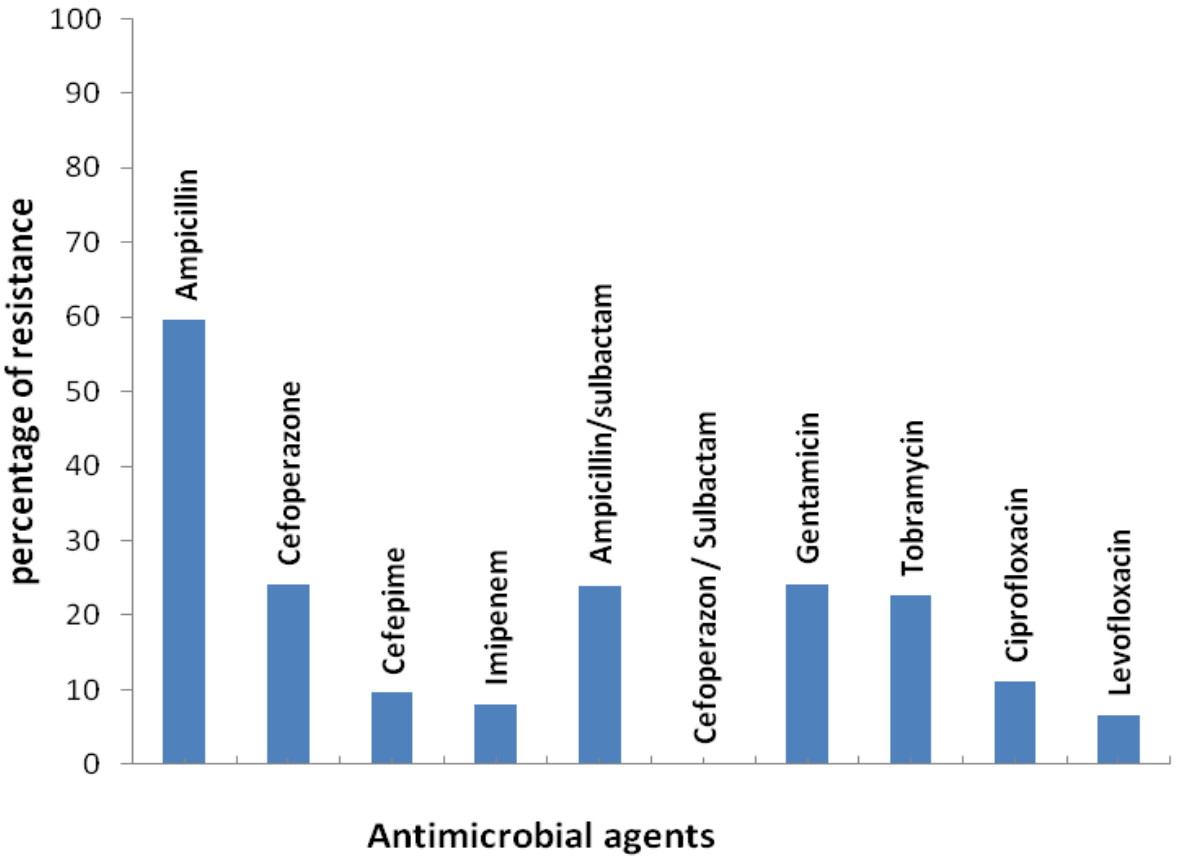

Figure 1. Percentage of Antimicrobial susceptibility pattern of *Proteus.*

Table 4. Integrons and characterization of gene cassettes.

Strain accession number	No of strain and source	*intI1*	*intI2*	*intI3*	size	Gene cassettes
KM386400	19 (Wound M)	+	-	-	726	*dfrA15*
KM386399	11 (endotracheal. M)	+	-	-	737	*dfrA15*
KM386403	31 (Urine D)	+	-	-	1665	*(dfrA17) -(aadA5)*
KM386405	27(Bile M)	+	-	-	1665	*(dfrA17) -(aadA5)*
KM386402	30 (Urine D)	+	-	-	1665	*(dfrA17)- (aadA5)*
KM386404	16 (Urine D)	+	-	-	1665	*(dfrA17)- (aadA5)*
KM386407	43 UP (human St D.)	-	+	-	857	(sat2)
KM386401	66 (cow Stool, D)	+	-	-	726	*dfrA15*
-	4 (Ear M)	-	+	-	156	No gene
-	11 (Endotrach. M)	-	+	-	156	No gene
-	43 (Stool D) down	-	+	-	156	No gene

D: Damietta, M: Mansoura, Strains: 9 and 10 (Wound D), 6(Ear D), 17(Stool D), and 5 (wound M) were devoid of integrons.

Table 5. Relationship between different antibiotic resistance pattern, and integron gene cassette of some related highly resistant isolates of *Proteus mirabilis* and source of isolation.

Strain accession number	Strain No.	Origin	Isolation center	Sample source	Sex	Antibiotyping profiles										Pattern No.	Integron components	Size
						AMP	CEP	FEP	IPM	SAM	SCF	CN	TOB	CIP	LEV			
KM386399	11	Human	GEC	Endo tracheal aspirate	F	R	R	S	S	S	S	R	R	S	S	10	*dfrA15*	737
KM386400	19	Human	MUH	Wound	M	R	R	S	S	S	S	R	R	S	S	10	*dfrA15*	726
KM386403	31	Human	DCJL	Urine	F	R	R	S	S	R	S	R	R	I	R	18	*(dfrA17) -(aadA5)*	1665
KM386405	27	Human	GEC	Bile aspirate	M	R	R	S	S	R	S	R	R	I	R	18	*(dfrA17) -(aadA5)*	1665
KM386402	30	Human	DSH	Urine	F	R	S	S	S	R	S	R	I	R	I	20	*(dfrA17)- (aadA5)*	1665
KM386404	16	Human	GGHD	Urine	F	R	I	S	S	R	S	R	R	I	S	13	*(dfrA17)- (aadA5)*	1665

epidemiological relatedness between different *Proteus* isolated from human animal and regional sources. Two adjacent Egyptian governorates were selected. Out of 500 samples, a total of 70 isolates of *Proteus* species were isolated from patients, stools of healthy humans and animals in Dakahleia and Damietta governments. A similar Egyptian work could not be traced in the available Egyptian literature.

Comparative studies were directed towards conventional methods of typing including biochemical reactions and antibiogram typing methods. It was also of interest to trace out the differences, similarities or relationships between different isolated strains by using integron finger printing of the isolated strains.

The objectives of epidemiological studies are to identify the source of disease, means of transmission, scale of distribution, epidemic and pandemic potential (or extent). Detection of asymptomatic carriers or reservoirs, and other factors associated with the spread of the disease are of great importance. To accomplish such objectives, there must be some means of characterizing the specific strain of the disease agent that is responsible, so that the past, present and future dissemination of the causative strain can be tracked (Bricker, 2011). The present study reveals that 14% of the examined samples were found to contain proteus sp. Out of them,

88.6% were classified as *P. mirabilis* indicating a high percentage as compared with 11.4% of *P. vulgaris.* It appears that the former is more invasive than the latter through the investigated region.

Antimicrobial susceptibility testing is a common practice in the clinical microbiology laboratory. The resultant antibiogram indicates the pattern of *in vitro* resistance or susceptibility of an organism to panel antimicrobial agents. The distribution of antimicrobial resistance among *Proteus* isolates showed that ampicillin was inactive against most of the tested isolates, where forty isolates (57.1%) were resistant to ampicillin. Such finding may be due to its extensive use in the treatment of different infectious diseases, while combination of cefoperazone (75µg) –sulbactam (30 µg) SCF was the most effective antibiotic combination where all the tested isolates were sensitive. The incidence of resistance to extended spectrum β-lactams and cephalosporins was previously observed. A higher percentage of resistance to ampicillin was found in the study of Bahashwan and El Shafey (2013) in Kingdom of Saudi Arabia (KSA) where more than 80 % of *Proteus* isolates were resistant to ampicillin. While similar result of resistance to ampicillin was found by Wong *et al*., 2013. Seventeen isolates (24.3%) were resistant to cephalosporins. A conforming result (26% to cefotaxime) was recorded by Yan-yan et al. (2012). However, a higher percent of resistance (more than 80% to cefpiramide and cephalothin) was reported by Bahashwan and El Shafey (2013). A lower percent of resistance (9.2% to ceftriaxone and 7% to cefepime) was reported by Maraki et al. (2012). β-lactam antibiotics are the most widely used antibiotics in clinical practice. This family of drugs is favored due to their high clinical efficacy, a broad spectrum of activity and safety. Due to frequent use of these antibiotics, bacteria have acquired a number of resistance mechanisms against these drugs (Sandanayaka and Prashad, 2002)

Resistance to aminoglycosides (gentamicin and tobramycin) was also observed. Sixteen isolates were resistant (22.85%). A higher percent of resistance to gentamicin (62%) was reported by Saleh and Hatem (2013). In contrast to the results of Yoon et al. (2011), who conducted their research on acute urinary tract infection in children from Korea. He showed that 100% of the isolates were sensitive to both gentamicin and tobramycin.

Aminoglycosides are commonly used antimicrobial agents in the treatment of infections by both Gram-negative and Gram-positive organisms. Aminoglycosides bind to the ribosomes and thus interfere with protein synthesis. Resistance to these antimicrobial agents is widespread, with more than 50 aminoglycoside-modifying enzymes already described (Shaw et al., 1993). The resistance of *Proteus* isolates to quinolones (ciprofloxacin and levofloxacin) was also observed. Ten samples (14.3%) were resistant, and ten samples were intermediate to either ciprofloxacin or levofloxacin or both. A similar result of resistance was observed by Orhiosefe et al. (2009) and Adamus-Bialek et al. (2013) as reported in their research on *P. mirabilis* strains isolated from clinical samples from Sweden and Poland. In contrast, a higher percent of resistance to ciprofloxacin (40%) was concluded by Kwiecińska-Piróg et al. (2013). Referring to the resistance pattern, it appears that the presence of four imipenem resistant isolates with their susceptibility to cefoperazone-sulbactam, suggests a sort of sulbactam-susceptible carbapenemase. This valuable information may constitute a simple method for their detection like ESBLS. In addition, other types of betalactamases can be easily interpreted from cephalosporin and ampicillin sensitivity tests.

For a more proper discrimination between resistant strains, molecular studies were applied. The term molecular epidemiology was firstly used to describe DNA-based methods to type, or fingerprint, strains of infectious microbes. During the past 15 years, many molecular methods have been adopted for use as typing schemes to assist the course of epidemiological investigations. More recently, molecular techniques have been applied to detect infectious agents in clinical or environmental samples providing greater sensitivity than was possible with conventional culture methods in the laboratory. Therefore, molecular epidemiology includes four applications of molecular techniques in infectious diseases: (1) to demonstrate relatedness between strains for epidemiologic investigations (2) to facilitate diagnosis, (3) to identify the agents of syndromes whose causes are unknown and (4) to identify genes involved in pathogenesis of infection and/or disease (Stout et al., 1992).

Integrons have been identified as a primary source of resistance genes and were suspected to serve as reservoirs of antimicrobial resistance genes within microbial populations (Navia et al., 2004). The role of integrons and gene cassettes in the spread of antibiotic resistance has been well-established (Kaczmarczyk et al., 2011). Class 1 integrons are the dominant type detected in clinical isolates and are most correlated with antibiotic resistance; therefore they have been comprehensively studied. Class 2 integrons are the second major type of integrons obtained from clinical isolates. Integrons, as a natural cloning and expression system, can capture exogenous gene cassettes by site-specific recombination and ensure the expression of the genes within them. Therefore, they play important roles in the acquisition and lateral transfer of antibiotic resistance genes (Wei et al., 2013)

Through the present study, we tried to characterize class 1, 2 and 3 integrons and their gene cassettes conferring resistance to several classes of antibiotics in *Proteus* sp. isolates were collected from clinical patients, human stool, and animals. Sequence analysis of the class 1 integron variable region revealed that the 1665 bp amplicon harbored gene cassettes (*dfrA17*) -(*aadA5*) conferring resistance to trimethoprim and aminoglycosides respectively. The 1496 bp amplicon harbored (*aac(3)-Id*)-

(*aadA7*) encoding aminoglycoside acetyltransferase and aminoglycoside adenyltransferase, respectively. Finally, the 726-737 bp amplicon contained the dfrA15 gene for trimethoprim resistance was found in three isolates. Sequence analysis of the class 2 integron variable region revealed only one isolate is having 857 bp amplicon carried (*sat2*) streptothricin acetyltransferase gene. Wei et al. (2013) reported that in 96 class 1 integron-positive strains, variable regions were successfully amplified in 70 isolates. Eight different gene cassette arrays were detected. The most prevalent gene cassette arrays were *aadB-aadA2* and *dfrA17-aadA5*, which were detected in 37 and 17 isolates, respectively. In the same study, in 101 class 2 integron-positive strains, variable regions were successfully amplified in all of them. Four different gene cassette arrays were detected. The most prevalent of these gene cassette arrays was *dfrA1-sat2-aadA1*, which was detected in their isolates. It worth mentioning that the absence of integrons in 4 of the tested resistant strains indicates that resistance genes can be located apart from integrons.

Comparing the results of integron components with antibiotic resistance pattern number, it appears that class 1 integron components were found in strains No 11&19. Such strains were belonging to antibiotic resistance pattern No10. They were isolated from a wound in Mansoura and bile aspirate from the same governorate. Such data Indicates with absolute confidence that they are one and the same strain running through hospital infection in Mansoura. Two other coincident strains including one isolate obtained from urine samples in Damietta (No31) and one from bile aspiration in Mansoura (No27). They have the same antibiotic sensitivity pattern No18. Such findings undoubtedly confirm the liability of transfer of strains from one governorate to the other. Approaching similarity, Strains No 16 and 30 are strongly related to strains No 30 and 27 with a liability to be transferred through a hospital infection in Damietta. It appears that minor changes in the antibiotic sensitivity pattern may be related to different antibiotic treatment approach even in the same governorate.

Molecular analysis of integron components confirms the presence of identical characteristics between some resistant strains and declares at least two pairs of sporadic strains are running through hospital infection cases or between the two adjacent governorates under investigation.

Such studies conforms with those of Michelin et al. (2008), who worked on 35 strains from different hospitals. The authors concluded that the only epidemiologically related strains were those strain isolated from one and the same patient occurring few days after endogenous infection of the patient himself.

Conclusion

Some of the studied resistant strains of *P. mirabilis* are epidemiologically related. Two pairs of identical strains suggest the possibility of hospital infection and a third pair suggesting the possibility of strain transfer from one adjacent governorate to the other.

ACKNOWLEDGMENTS

This work was performed at Microbiology Department, Faculty of Pharmacy, Mansoura University, Egypt.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Adamus-Bialek W, Zajac E, Parniewski P**,** Kara W (2013). Comparison of antibiotic resistance patterns in collections of *Escherichia coli* and *Proteus mirabilis* uropathogenic strains. MolBiol Rep. 40(4):3429-3435.

Bahashwan SA, El Shafey HM (2013). Antimicrobial resistance patterns of *Proteus* isolates from clinical specimens. Eu. Sci. J. 9(27):188-202.

Bricker BJ (2011). Present and future of molecular technology applications for the epidemiology of bacterial diseases. J Anal. Bioanal. Techniques S10:001. Doi:10.4172/2155-9872.

Chen CY, Chen YH, Lu PL, Lin WR, Chen TC, Lin CY (2012). *Proteus mirabilis* urinary tract infection and bacteremia: risk factors, clinical presentation, and outcomes. J. Microbiol. Immunol. Infect. 45: 228-236.

CLSI (2010). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard, CLSI document M7-A6. CLSI 2010.

Crichton PB (1996). Enterobacteriaceae: *Escherichia*, *Klebsiella*, *Proteus* and other genera. In Mackie & McCartney Practical Medical Microbiology, 14th ed (Collee, J. G., Fraser, A. G., Marmion, B. P. & Simmons, A., Eds), 361–84. Churchill Livingstone, Edinburgh, UK.

Kaczmarczyk M, Walsh C, Slowey R, Leonard N, Fanning S (2011). Molecular characterization of multidrug-resistant *Escherichia coli* isolates from Irish cattle farms. Appl. Environ. Microbiol. 77:7121-7127.

Kwiecińska-Piróg, J, Krzysztof S, Katarzyna Z, Eugenia G (2013). The Assessment of *Proteus mirabilis* susceptibility to ceftazidime and ciprofloxacin and the impact of these antibiotics at subinhibitory concentrations on *Proteus mirabilis* biofilms. J Biomed Res Int. Article ID 930876, eight pages

Lipsitch M, Samore MH (2002). Antimicrobial use and antimicrobial resistance: a population perspective. Emerg. Infect. Dis. 8(4):347-354.

Maraki S, Samonis G, Karageorgopoulos DE, Mavros MN, Kofteridis D, Falagas ME (2012). *In Vitro* Antimicrobial susceptibility to isepamicin of 6,296 Enterobacteriaceae clinical isolates collected at a tertiary care university hospital in Greece. Antimicrob Agents Chemother. 56(6):3067-3073.

Michelin L, Muller G, Zacharias J, Delamare APL, da Costa SOP, Echeverrigaray S (2008).Comparison of PCR-based molecular markers for the characterization of *Proteus mirabilis* clinical isolates. Braz. J. Infect. Dis. 12(5):423-429.

Navia MM, Ruiz J, Vila J (2004). Molecular characterization of the integrons in *Shigella* strains isolated from patients with traveler's diarrhea. Diagn. Microbiol. Infect. Dis. 48:175-179.

Orhiosefe O, Lawrence O, Patience U (2009). Increasing resistance to quinolones: A four-year prospective study of urinary tract infection pathogens. Int J Gen Med. 2: 171-175.

Saleh AB, Hatem MES (2013). Antimicrobial resistance patterns of *Proteus* isolates from clinical specimens. Eu. Sci. J. 9(27):188-202.

Sandanayaka V, Prashad A (2002). Resistance to beta-lactam antibiotics: structure and mechanism based design of beta-lactamase inhibitors. Curr.Med. Chem. 9: 1140-165.

Shaw KJ, Rather PN, Hare RS, Miller GH (1993). Molecular genetics of aminoglycosides resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol. Rev. 57: 138-163.

Stout JE, Yu VL, Joly J (1992). Potable water as a cause of sporadic cases of community-acquired Legionnaires disease. N Engl. J. Med. 326: 151-55.

Wasfi R, Abd El-Rahman OA, Mansour LE, Hanora AS, Hashem AM, Ashour MS (2012). Antimicrobial activities against biofilm formed by *Proteus mirabilis* isolates from the wound and urinary tract infections. Indian J. Med. Microbiol. 30: 76-80.

Wei Q, Hu Q, Li S, Lu H, Chen G, Shen B, Zhang P, Zhou Y (2013). A novel functional class 2 integron in clinical *Proteus mirabilis* isolates. J. Antimicrob. Chemother. 69 (4): 973-76.

Wong MH, Wan HY, Chen S (2013). Characterization of multidrug-resistant *Proteus mirabilis* isolated from chicken carcasses. Foodborne Pathog Dis. 10(2):177-781.

Yan-yan H, Jia-chang C, Rong Z, Hongwei Z, Qian S, Gong-Xiang C (2012). The emergence of *Proteus mirabilis* harboring blaKPC-2 and qnrD in a Chinese hospital. Antimicrob Agents Chemother. 56(5): 2278-2282.

Yoon JE, Kim WK, Lee JS, Shin KS, Ha TS (2011). Antibiotic susceptibility and imaging findings of the causative microorganisms responsible for acute urinary tract infection in children: a five-year single center study. Korean J. Pediatr. 54(2):79-85.

Zhang HM, Shi L, Li L, Guo SY, Zhang XM, Yamasaki S, Miyoshi S, Shinoda S (2004). Identification and characterization of class 1 integron resistance gene cassettes among *Salmonella* strains isolated from healthy humans in China. Microbiol. Immunol 48: 639-645.