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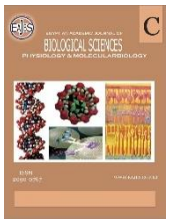

Role of Integrons 1 and 2 in *Proteus spp*. and Evaluation of Some Antibiotic's Resistance Genes

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ABSTRACT

A total number of 150,86 samples (57.3%) of samples were females, and 64 (42.7%) of samples were males, so from total specimens of 150 showed significant bacterial growth. The most common pathogen was *proteus spp* (50%), *Staphylococcus spp* (8.3%) *Klebsiella pneumoniae* (11.7 %), *streptococcus spp* (16.7%), *Serratia marcescens* (13,3%), Antibacterial susceptibility test was conducted for 30 *proteus spp* isolates against 20 commonly used antibacterial agents by using the disk diffusion method, the highest rate of resistance was seen with Ampicillin 30/30(100%) followed Amoxicillin- clavulanic Acid 29/30 (97.5%) and colistin 27/30 (90 %) and the low rate resistance was seen with Meropenem 0/30 (0%) and imipenem was 1/30 (7.5%), the results showed presence of integron-1 in *Proteus spp* isolates, from the 30 (100%) isolates of*Proteus spp was* 5 (16.65 %) is positive and integron-2 was (83.25 %) 25 positive for 30 *proteus spp* isolates. The results showed that the (blaOXA-1) gene was detected in*Proteus spp* isolates, from the 30 (100%) isolates 9 (30.3 %) were positive. where have blaTEM gene, the result was (40%) positive, and bla SHV gene was found in 2 isolates (6.66%), and finally the bla AMP-c gene was positive in 6 isolates (19%).

INTRODUCTION

Proteus spp. are Gram-negative rods of the order Enterobacterales. These species are common in the environment, soil, and water, and are part of the natural bacterial flora of human and animal intestinal tracts. thought to be caused by fecal contamination, The biochemical properties shared by the genera Proteus do not contain Arginine dihydrolase or Lysine decarboxylase. Ornithine deaminase and Phenylalanine deaminase positivity Growth on potassium cyanide culture media (KCN) positive for d-Glucose from acid test but not from acid melibiose and Nitrite to Nitrate conversion Oxidase production is negative, ONPG production (O-Nitrophenyl-β-D- glucopyranoside) is positive, and pectate utilization is negative, There are six species in the Proteus genus (P. mirabilis, P. vulgaris, P. penneri, P. cibarius, P. terrae, and P. hauseri), as well as three genomospecies (Rahman *et al*., 2017; CLSI,2022) type, Proteus spp identified as producers of extended-spectrum beta-lactamase (ESBL) in 1987.

Proteus species demonstrated susceptibility to beta (β)-lactam antibiotics (Tahmourespour *et al*.,2011) The dissemination of extended-spectrum β-lactamases, which exhibit activity against extended-spectrum cephalosporins, has begun to occur. This includes the frequent occurrence of TEM-type derivatives, as well as other enzymes. The development of resistance to beta-lactam drugs is attributed to the presence of enzymes encoded by both chromosomal and plasmid DNA.

MATERIALS AND METHODS

Samples Collection And Bacterial Identification:

A total number of 150(different types of samples) were collected from patients admitted to AL-Zhraa Hospital and AL-sadder Medical City AL-manadra General Hospital AL-mshkabe General Hospital AL-Najaf Alashrf Teaching Hospital in AL- Najaf Governorate, during the period from November 2022 to Jun 2023. All samples were collected in a way to avoid any potential contamination, Swabs were taken and closed until transported to the advanced Microbiology laboratory / Al-Furat al Furat Aswat Technical University College of Health and Medical technique / Kufa and culturing on different media for 24 hours at cultivate 37 °C for bacterial diagnosis (Kashfi *et al*.,2017).

Antibiotic Susceptibility Test:

Muller Hinton agar was prepared, sterilized in the autoclave and poured into petri dishes, then Antibiotic resistance *Proteus spp* isolates were streaked by sterile swab on a petri dish and placed antibodies disc and incubated the dishes at 37 ° C for 24 hrs, the diameter of inhibition zones was measured using a meter ruler (CLSI,2022).

Molecular Techniques Extraction of Genomic DNA:

Genomic DNA was extracted by using a method of (Yang *et al* .,2008).

Molecular Identification:

Gel electrophoresis was used to determine DNA via UV trans illuminator, the primer was planned by Alpha DNA company, Canada as in Table (1).

Table 1: Gel electrophoresis used to determine DNA via UV trans illuminator.

Name ofgenes	Oligo-Sequence (3′→5′)	ProductSize (bp)	Reference
Bla TEM	F: TCAACATTTTCGTGTCGCCC R: AACTACGATACGGGAGGGCT	566	Yang, *et al.,* 2020
Bla SHV	F: CTTTACTCGCCTTTATCG R: TCCCGCAGATAAATCACCA	827	Kolar *et al.,* 2020
bla ampC	F: TTCTATCAAMACTGGCARCC R: CCYTTTTATGTACCCAYGA	550	Shahein *et al.,* 2021
Int M1-U *IntM1-D*	F 5'-ACGAGCGCAAGGTTTCGGT-3' R: 5'-GAAAGGTCTGGTCATACATG-3'	500	Mohamed Adel El-Sokkary *et al*.,2015
IntM2-U *IntM2-D*	F: 5'-GTGCAACGCATTTTGCAGG-3' R: 5'-CAACGGAGTCATGCAGATG-3	750	Mohamed *et al.,* 2015
Bla oxa 1	F:ATATCTCTACTGTTGCATCTCC R: AAACCCTTCAAACCATCC	650	Samyyia Abrar, 2019

PCR Thermo-Cycling Conditions:

The PCR tubes were placed on the PCR machine and the right PCR cycling programparameters conditions were installed as in Table (2).

Table 2: Amplification conditions of genes were used by PCR reactions.

GeneName	Temperature(◦c)/Time					Cycles Number
	Initial Denaturation	Cycling conditions			Final Extension	
		Denaturation	Annealing	Extension		
Int1	94/10min	94/30sec	53/30sec	72/30sec	72/5min	30
Int2	94/10 min	94 / 30sec	52 /1 min	72 / 30sec	72/5 min	30
Bla-oxa-1	94/5min	94/30sec	56/30sec	72/1min	72/5min	40
Bla-SHV	94/ 5min	95 / 30sec	50/35sec	72/1min	72/5min	30
Bla-TEM	94/5min	94/30sec	56/30sec	72/1.5sec	72/7min	30
Bla-AMP-C	94/5min	94/30sec	50/40sec	72/45sec	72/10min	30

RESULTS AND DISCUSSION

The characteristics of the study samples were mentoied in Table (3), the patients' samples of proteus spp infection were obtained from the hospitals in Al- Najaf province, A total number of samples 150 (100%),86 (57.3%) samples were female, 64 (42.7%) samples were male, so a total specimens of 150 showed significant bacterial growth. so, total specimens of 233 (93.2%) showed significant bacterial growth in 153 (65.7%) female specimens, 56 (24.0%) from male specimens and 24 (10.3%).

Table 3: Characteristics of the study sample

Variable	Study sample (N=150)			Statistical test	P-Value
	Groups	No.	%		
Age (years)	10-20	16	10.7	χ^2: 750.0	**<0.001**
	21-30	20	13.3		
	31-40	23	15.3		
	41-50	25	16.7		
	51-60	22	14.7		
	>60	44	29.3		
Gender	Male	86	57.3	χ^2: 150.0	**<0.001**
	Female	64	42.7		
Education	Primary	86	57.3	χ^2: 300.0	**<0.001**
	Secondary	54	36		
	University	10	6.7		
Residence	Rural	95	36.7	χ^2: 145.7	**<0.001**
	City	55	63.3		

Proteus spp infections are prevalent bacterial infections that often affect most components of the body, it is considered the third most common cause of UTI infection then gastrointestinal infections and cause significant morbidity and considerable mortality that affects a large group of people each year worldwide (Mishra *et al*., 2020). It is more common in women than in men because of the anatomical proximity of the urethra to the gut opening and because the normal habitat of Proteus spp is the gastrointestinal tract (Poirel *et al*., 2010; Mishra *et al*., 2020; Fazly *et al*., 2021).

Identification of *Proteus spp* and Other Species In Samples:

Samples were cultured on blood agar and MacConkey agar to see the swarming phenomenon.The biochemical tests and Vitek 2 system for the bacterial final diagnosis were investigated in the laboratory Table (4), from (150) samples (60) were for *Proteus spp,* and other bacteria were used as control positive (Konate *et al.,* 2017; Wellington *et al* ., 2013).

Table 4: Bacterial species that isolate among culture samples.

Pure culture bacteria	Number	Percent (%)
Proteus spp.	30	50%
Staphylococcus spp.	5	8.3%
Streptococcus spp.	10	16.7%
Klebsiella pneumonia	7	11.7%
Serratia marcescens	8	13.3%
Total positive isolates	60	100%
Chi-square & P. value	χ^2: 240.00, P: <0.001	

Antibiotic Susceptibility of *P.mrabilis* Isolates:

Antimicrobial susceptibility test towards twenty antibiotics was determined using agar disc diffusion test (Kirby-Bauer method) according to the Clinical Laboratory Standards Institute (CLSI) guidelines (2022).in this study, revealed that (30) isolatesof Proteus spp showed a variable level of resistance to antibiotics, as shown in the Table(5) (Al-Jumaily, 2016).

Table 5: Antibiotic susceptibility test for 30 *P.mirabilis* isolates

Type of antibiotics class (subclass)	Antibiotic disk	No. (%) antibiotic Resistance	
		Resistance	Sensitive
Fluoroquinolones	Ciprofloxacin	18 (70%)	12(30%)
	Norfloxacin	6(15 %)	24 (85%)
Quinolones	Nalidixic Acid	14 (47.5%)	16 (52.5%)
Nitrofurans	Nitrofurantoin	23 (82.5%)	7 (17.5%)
β-lactams cephalosporines	Ceftazidime	19 (72.5%)	11 (27.5%)
	Ceftriaxone	18 (70%)	12 (30%)
	Cefixime	21 (52.5%)	11 (47.7%)
	Cephalothin	21(77.5%)	9(22.5%)
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	Cephalothin	21(77.5%)	9(22.5%)

Misuse and overuse of antibiotics by healthcare professionals and the public are among the many factors contributing to an increase in antimicrobial resistance rates

(Konate *et al.,* 2017). Inadequate surveillance systems and dependence on reliable microbiological techniques also contribute to the improper prescription of antibiotics (Wellington *et al* ., 2013) According to (Shabeeb *et al* .,2016), *Proteus spp* produces ESBLs, carbapenemase, and AmpC as part of its antibiotic resistance mechanism. These enzymes pose a high risk to the public and are responsible for numerous outbreaks. ESBLs are also expensive, increase the length of hospital stays, and cause more complications (El-Gamasy *et al.,* 2017). Our results suggest that those antibiotics shouldnot be used in the treatment of *Proteus spp* infections, as it will lead to failure of therapy. the highest rate of resistance was seen with Ampicillin 30/30(100%) followed by Amoxicillin-clavulanic acid 29/30 (97.5%) and Colistin 27/30 (90 %). Doxycycline 26 /30 (90%), Trimethoprim-sulfamethoxazole 24/30 (85%), Nitrofurantoin 23/30 (82.5%), Tetracycline 22/30 (80%), Cephalothin 21/30 (77.5%), Ceftazidime 19/30 (72.5%), Ceftriaxone, Ciprofloxacin 18/30 (70%), Cefixime 11/30 (52.5%), Nalidixic Acid 9 /30 (47.5%), whereas the low rate of *proteus spp* resistance was seen with Tobromycin 8/30 (27.5%), Gentamicin 6 / 30 (20%), Amikacin 4/30 (17.5%), Norfloxacin 3/30 (15%), Piperacillin / Tazobatam 2/30 (7.5%), Impenem 1/ 30 (2.5 %), Meropenem 0/30 (0.0 %) (Al-Jumaily, 2016; Attallah *et al* ., 2020). The problem in treating patients caused by the rise in antibiotic resistance raises the mortality rate. Meropenem, imipenem, amikacin, gentamicin, norfloxacin, and Piperacillin / Tazobactam had the lowest rates of resistance discovered in this study, this may be attributable to the poor usage of these antibiotics in Al-Najaf hospitals. Meropenem was the first place it was the most effective and sensitive drug against *Proteus spp* isolates in the current study, This result agreed with those who found the sensitivity to this drug 100% And also resembles (Sun *et al*., 2020), who documented that the resistance of *Proteus spp* was 4.5%, while this result disagrees with (Mirzaei *et al* ., 2021) observed that *Proteus spp* bacteria had a 47.1% resistance rate to this antibiotic, Furthermore (El-Tarabili *et al*.,2022) indicated that the resistance of all of its isolates to meropenem was 0%. The occurrence of resistance within *Proteus spp* to several antibiotics is an emerging problem, extremely complex and made UTIs management progressively more costly and challenging. Which limited the choices for selecting the appropriate drug for the treatment of *Proteus spp* the high incidence of *Proteus spp* infections, has been known widely for its contribution to the worldwide dissemination of multidrug resistance (MDR). When a bacterial strain is resistant to at least three classes of antibiotics defined as MDR bacteria (Li et al., 2021). The present study demonstrated that all *Proteus spp* isolates (100%) were MDR showing resistance to a minimum of three classes of antibiotics.

Detection of Genes That Responsible For Antibiotic Resistance in *Proteus spp:*

The results showed that the bla OXA-1 was detected in isolates (Fig.1), from the 30 (100%) isolates 9 (30 %), The results also showed that the bla TEM gene was detected in *Proteus spp* isolates (Fig.2), from the 30 (100%) isolates 12 (40%) and isolates have bla SHV gene in 2 samples just 2 (6.66%) (Fig.3). The results also displayed that bla AMP-c was detected in *Proteus spp* isolates (Fig.4), from the 30 (100%) isolates 6 (19 %) and the isolates found having a genomic structure culled integron and in this study (Fig.5), we investigate type1 and 2, the results revealed present of int-1 in 5 isolates (16.65%) and int-2 found in 25 isolates (83.25%) (Li *et al.,* 2021).

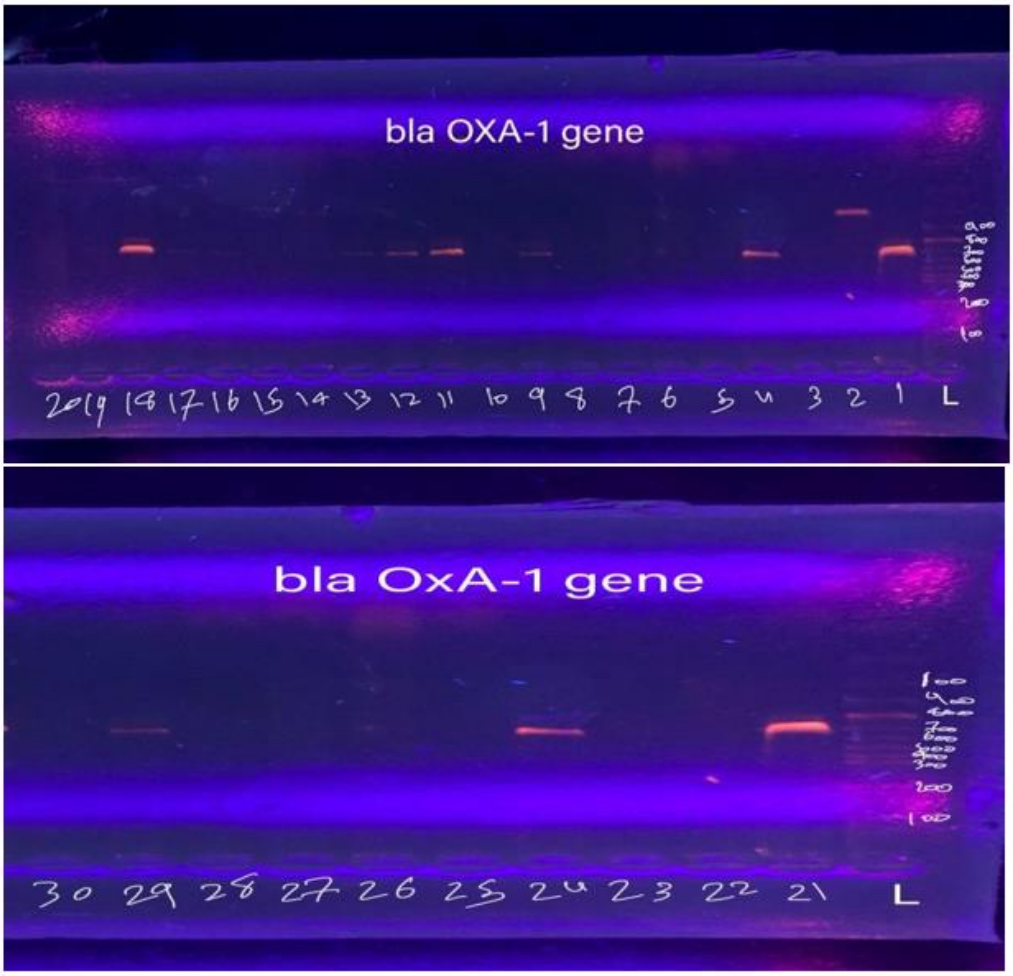

Fig.1: Agarose gel with ethidium bromide staining of mono-plex PCR amplified product from extract DNA of *Proteus spp* isolates with (*bla OXA-1*) gene primers, Lane (L) DNA molecular size marker (`100-bpladder) , Lane (1,4,9,11,12,18,21,24,29) show positive results.

Fig.2: Agarose gel with ethidium bromide staining of mono-plex PCR amplified product from extract DNA of Proteus spp isolates with (blaTEM) geneprimers, Lane (L) DNA molecular size marker (100-bpladder), Lane (1,3,4,5,6,7,11,15,23,24,26,27) show positive results.

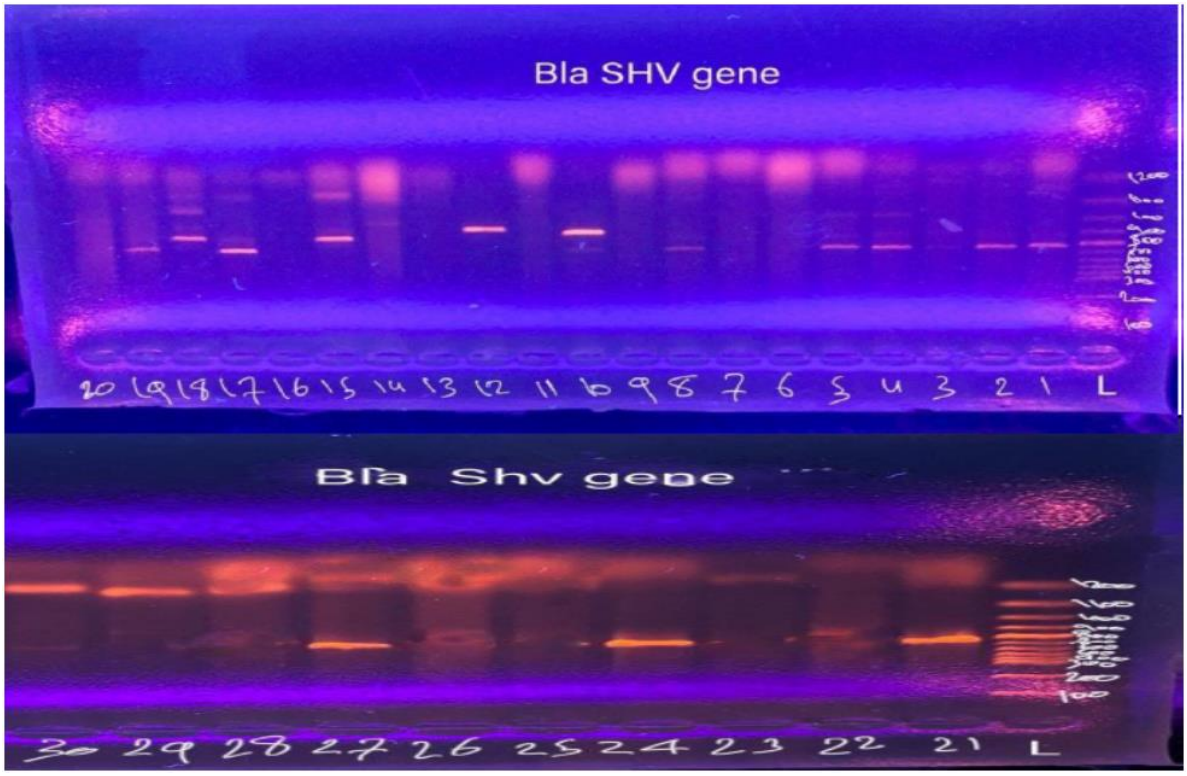

Fig. 3: Agarose gel with ethidium bromide staining of mono-plex PCR amplified product from extract DNA of *Proteus spp* isolates with (*blaSHV*) geneprimers, Lane (L) DNA molecular size marker (`100-bpladder), Lane (10,12) shows positive results**.**

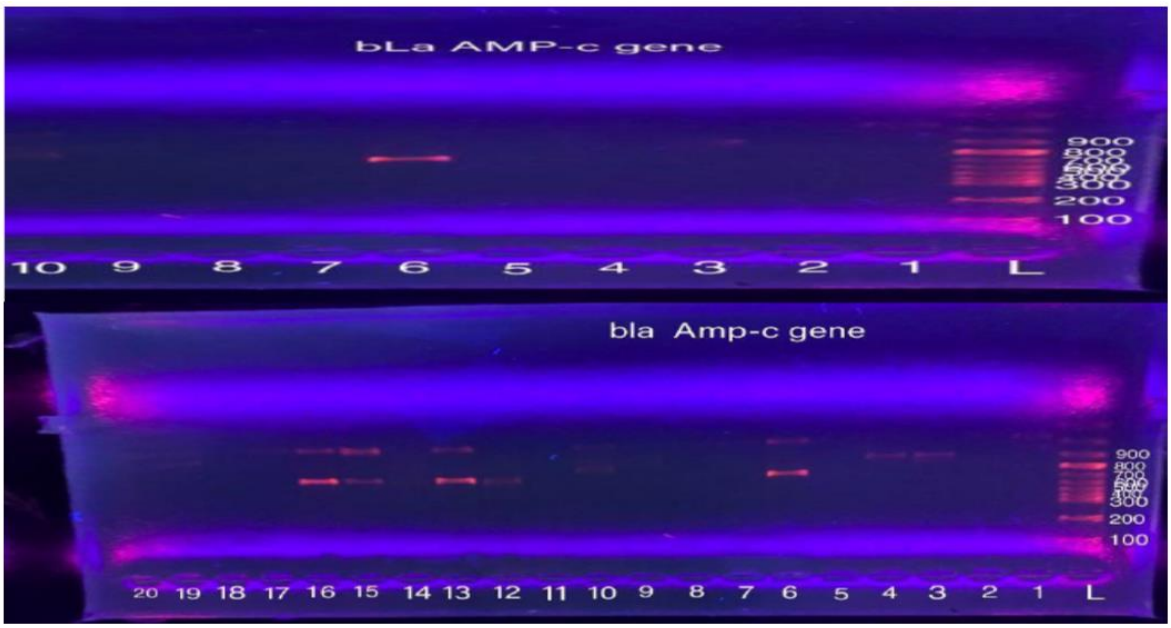

Fig. 4: Agarose gel with ethidium bromide staining of mono-plex PCR amplified product from extract DNA of *Proteus spp* isolates with (*bla AMP-c*)gene primers, Lane (L) DNA molecular size marker (`100-bpladder), Lane (1,3,4,5,6,7,11,15,23,24,26,27) show positive results.

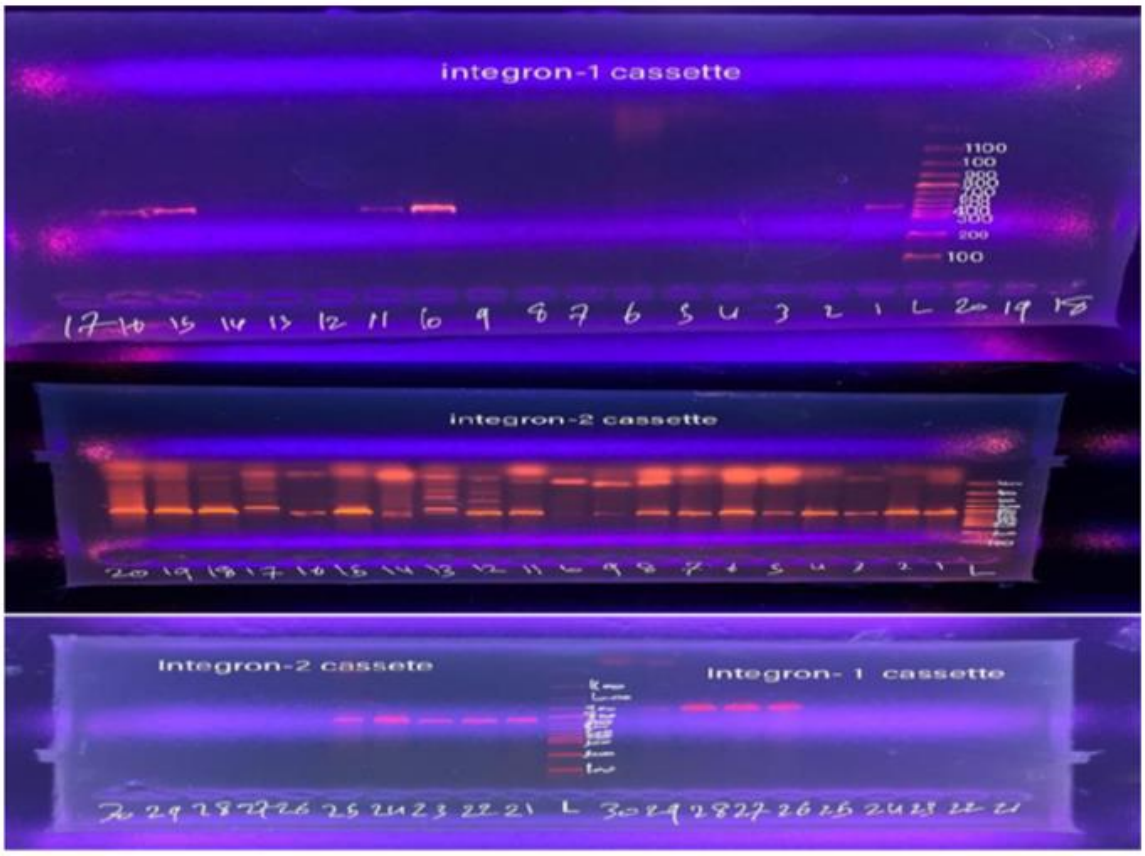

Fig. 5: Agarose gel with ethidium bromide staining of mono-plex PCR amplified product from extract DNA of *Proteus spp* isolates with (*int-1*)(int-2) cassette gene primers, Lane (L) DNA molecular size marker (`100-bpladder), Lane (from 1 -25 in int-2) shows positive results and (1,10,11,15,16) showspositive results (int-1) cassette gene.

ESBLs are widely distributed among gram-negative pathogens and are usually encoded by genes carried on plasmids ESBL distributions are highly variable. *Proteusspp* has been shown to produce TEM-type ESBLs, according to some studies. When compared to the TEM-1 type, TEM-72 ESBL, discovered in *Proteus mirabilis* in Italy in 2000, showed four amino acid substitutions (Q39K, M182T, G238S, and E240K). French scientists identified TEM-92 ESBL from *Proteus spp* in 2001. (Li *et al.*, 2021) many multidrug-resistant (MDR) Gram-negative bacteria (24). According to our results, the (bla TEM) gene has the highest prevalence in *Proteus spp* strains and thisis consistent with (Meshref *et al* .,2021). The bla TEM is one of the genes that are responsible for hydrolyzes penicillins and many cephalosporins. Antibiotic resistance among patients infected with *Proteus spp* is a big issue, worsening prognoses and perhaps reducing the effectiveness of treatments. This is largely because of the widespread availability and careless application of antibiotics in many regions. Thus, as mentioned by (Meshref *et al* .,2021) and (Hamid et al .,2020), to enhance outcomes, treating physicians must recognize the presence of MDR organisms and their associated risk factors, such as hospitalization, and inappropriate use of antibiotics. Most of these patients had been dealing with no infection for quite some time,and it took a very long period of random Antibiotics taken. As a result, the widespread use of antimicrobial drugs creates a selective pressure that favors the evolution of resistance in microbes. All *Proteus spp* isolates tested positive for ESBL production and demonstrated resistance to at least one drug. Even more so than what was reported by (Hamid *et al* .,2020), this extremely high incidence of ESBLs in *Proteus spp* was observed.mPossible causes include variations

in sample composition, sample collection timing, and antibiotic dosing. The presence of multiresistant genes is a typical characteristic of ESBL-producing. *proteus spp* isolates. This pattern of resistance is like that observed by (Hamid *et al* .,2020), who found that their isolates were resistant not only to the beta-lactam groupbut also to other antibiotic classes like aminoglycosides, sulphonamides, and fluoroquinolones. Many different types of beta-lactamase genes were found in *Proteus spp* isolates in this investigation. These included blaTEM (40%), and lowest rate of bla SHV (6.66%),andblaOXA-1(30%). And bla AmpC genes (19%).. One isolate showed a favorable phenotype but tested negative for all beta-lactamase genes. This could be because other beta-lactamase genes were not tested. (Wu *et al* .,2012)

Identification and Characterization Of Gene Cassette Of Class 1 And 2 Integrons:

The horizontal transfer of genetic material within and between microbial genera has aided in the creation of novel antibiotic resistance characteristics. world-wide. Rapid and broad establishment of resistance, as well as similar patterns of resistance, have been observed on an increasing scale in phylogenetically diverse Gram-negative clinical isolates (Lu *et al* ., 2022). In addition to well-known bacterial mutation strategies, antibiotic genes are increasingly being captured in integron-borne cassettes, which provide an efficient mechanism for capturing and exchanging different resistance genes. Further analysis of the antibiotic resistance genes present in threaten resistant integrons positive *Proteus spp* isolates was conducted. In this study, *Proteus spp* isolates were discovered to have detectable Class 1-related integrons,with four having amplicons of 500 bp, and which agree with results of (16.65%) and (750 bp) (83.25%) for integron class-2 that is nearly like results of. Integrons 1 are critical in the spread of antimicrobial resistance via horizontal transmission. Their role in the spread of MDR Gram-negative bacteria has been established. They are screening participants in the current study. Integrons 1 were identified and described in MDR Gram-negative bacterial isolates in much research before.(de Curraize *et al.*, 2020). Class 2 integrons were found in (25) isolates (83.25%) of the 30 *Proteus spp* isolates that were studied. Antibiotic resistance genes were analysed in 25 integron-positive *proteusspp* isolates that were resistant to several antibiotics Categories. According to the present study, it seems that the limited ability of the second type of integrons to carry antibiotic resistance genes has changed due to gene mutations at the level of shapeand genetic arrangement, and this has given bacteria that have this type of integrons tremendous potential to resist a wide range of antibiotics. (Barns *et al*.,2021).

Conclusions

The research has shown that Proteus spp. Bacteria have become a significant health concern due to their acquisition of multiple antibiotic resistance genes like bla OXA-1gene and others. The study has revealed that infections caused by antibiotic-resistant *Proteus spp*. strains have a profound impact on human immunity. The ability of these bacteria to evade conventional treatment leads to persistent and severe infections, which can overburden the immune system.

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