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Biocontrol of *Proteus* causing Urinary Tract Infection: Morphological and Biochemical Studies

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Abstract Urinary tract infection (UTI) is a illness initiated by bacteria such as *Proteus* spp.Various antibiotic medications can be used to treat UTIs. Antibiotic resistance has unfortunately increased and is now a hazard to public health. The purpose of this study was to look for *Proteus* spp. that were multi-drug resistant (MDR).Fifty isolates of the *Proteus* spp. were isolated and then identified. strains isolated from patients in Mansoura University Hospitals, Mansoura City, Egypt, because of their importance for treating infections in people. By using morphological and biochemical methods. Their antimicrobial vulnerability to 11antimicrobial agents from 8 antimicrobial categories (aminoglycosides, penicillins, carbapenems, cephalosporins, fluoroquinolones, penicillins/ß-lactamase inhibitors, quinolone, diaminopyrimidines) was determined by the disc diffusion method, according to the reference of the medical and Laboratory tandards Institute. Isolates of *Proteus* spp. were classified as MDR in accordance with standardized international terminology, which was presented by the European Centre for Disease Prevention and Control. Medicinal plants proved high effectiveness in treating UTI with no side effects especially clove. the ethanolic extract of clove was the most active one with inhibition zones diameter ranged between (14mm – 25 mm).

keywords: Drug Resistant Bacteria, *Proteus* spp.Bacteria, Urinary tract infection

1.Introduction

Proteus spp. is an aerobic gram-negative bacteria. Their ability to go through morphological alterations of colonies is the basis for their names [1]. *Proteus* spp. possesses peritrichome flagella, making it motile. Swarming and an ammonia odour are two signs of a *Proteus* colony. Hauser coined the term "*Proteus*" for the first time in bacteriological nomenclature [2].

The *Proteus* habitat is widely dispersed throughout the world. *Proteus* is an opportunistic human pathogen that lives in the skin, oral mucosa, and gastrointestinal tracts of both humans and animals, as well as in excrement, soil, waterand plants. Temperatures between 10 and 43 °C are suitable for growing some *Proteus* strains. *Proteus* prefers a temperature of 25 °C. Swarming takes place between 20 and 37°C. *Proteus* frequently causes nosocomial urinary and septic infections [3].

There are three opportunistic human pathogen species of *Proteus*: *P.vulgaris*, *P.mirabilis*, and *P.penneri* [4]. Clinically, this organism most often causes urinary tract infections, especially in patients undergoing prolonged catheterization. *P.mirabilis* uses avariety of virulence factors, including urease and stone formation, fimbriae and other adhesins, iron and zinc acquisition, proteases and toxins, biofilm development, and pathogenesis regulation, to gain access to and colonieze the human urinary tract [5].

The urease enzyme produced by *Proteus* and other bacterial species causes the urine to become alkaline because it breaks down urea into ammonia and carbon dioxide. which leads to the formation of struvite stones, which

make up around 10-15% of kidney stones [6].

P.mirabilis has a lower susceptibility to imipenem and is obviously impervious to a range of antibiotics,including colistin. Additionally, resistance to non-lactams, such as those used to treat UTI infections, is also documented (e.g.,fluoroquinolones, nitrofurans).

High resistance ability is because of many factors, such as the acquisition of several antibiotic resistance genes, including carbapenemase genes, in *P. mirabilis* isolates [7].

The necessity for a continuous search for new antimicrobial chemicals has been driven by the issue of antibiotic resistance, which has restricted the use of inexpensive and outdated drugs. Since then, it has been noted that certain chemical substances, also known as MDR inhibitors or resistance modifying agents, have the capacity to alter the resistance phenotype in bacteria by collaborating with antibiotics in vitro. There may be a different treatment approach to the issue of bacterial resistance [8].

Furthermore, it is anticipated that plant chemicals with target sites different from those now used by antibiotics will be effective against infections that are resistant to drugs [9]. According to the World Health Organization, herbal treatments are prepared effectively using medicinal herbs. Nearly 80% of developed countries use herbal medicine extensively [10]. As some medicinal plants have antimicrobial action in curing disease for countless years, astoundingly, conventional medicine is used globally to manufacture antibiotics and advanced drugs [11].

It is concerning to see that a large percentage of *Proteus* spp. clinical isolates investigated in Egypt are multidrug resistant, and appropriate measures are needed to stop the spread of these strains. As some medicinal plants have antimicrobial action.

Materials and methods

Bacterial strains

Clinical *Proteus* isolates were collected from laboratory of Mansoura University Hospitals in 2021. These isolates were identified as *Proteus* spp. created on basic phenotypic organizations (morphology and pattern of gathering, biochemical reactions and growth at 42 ˚C).

Antimicrobial susceptibility test

Discovery of antimicrobial vulnerability in scientific isolates of *Proteus* was ended by disk diffusion method rendering to the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. The subsequent antibiotic disks from MAST Categories Ltd., Merseyside, UK, were used: Amoxicillin (AMX-AX,25µg), Amoxicillin&Clavulanic (AMC, 30µg), Amikacin (AK, 30µg), Ceftriaxone (CRO, 30µg), ceftazidime (CAZ, 30µg), Cefurexime (CXM,30µg), Meropenem (MeM, 10µg), Norfloxacin (NX-NOR, 10µg), Ciprofloxacin (CIP, 5µg), Nalidixic acid (NA, 30µg) and Trimethoprime (TR-SXT, 5µg). *Proteus* spp. was a control strain used for all antibiotics disks.

Detection of MDR isolates

Rendering to new standardized global document, the important of MDR in *Proteus* isolates were done [13], by the consequences of antimicrobial vulnerability of *Proteus* spp. to all antimicrobial mediators listed in Table (1).

Therefore, *Proteus* isolates, which have revealed resistance to at smallest 1 agent in ≥3 antimicrobial classes known as MDR.

Table (1): Antimicrobial classes and agents proposed for description of MDR in *Proteus* spp.

Antimicrobial categories	**Antimicrobial agents**
Aminoglycosides	Amikacin
Penicillins	Amoxicillin
Carbapenems	Meropenem
Cephalosporins	Ceftriaxone,ceftazidime, cefuroxime
Fluoroquinolones	Ciprofloxacin, norfloxacin
Penicillins/ß-lactamase inhibitors	Amoxicillin&clavulanic
Quinolone	Nalidixic acid
Diaminopyrimidines	Trimethoprime

Ethanolic plant extracts preparation:

The seven herbal plants were dried and pulverized into fine powder. The powdered material was stored in air tight sterile containers and protected from sunlight until required.

1. Ten grams (10g) of each dried ground plant material was mixed with 100 ml of 95% ethanol solvent in sterile conical flask, covered with foil paper and placed on a rotary shaker for 24 hours.
2. Then it was filtered through Whitman filter paper
3. The supernatant was collected and concentrated in vacuum for 15 min at 37°C using a rotary evaporator to make the last volume half of the novel volume (stock solution).
4. The concentration was then dissolved in 10 ml of 1% dimethyl sulfoxide (DMSO).
5. All extracts were sterilized by filtration through bacterial filter of pore size of 0.45µm using positive pressure, and then filtrate was kept at 4°C in the refrigerator till use.

Results

Table (2) recorded the data of antimicrobial susceptibility of 50 *Proteus* spp. isolates against 11 agents from 8 antimicrobial categories. The highest resistance was shown to ceftazidime (98%) and the highest susceptibility was shown to meropenem (90%).

Table (2): Antimicrobial susceptibility of 50 *Proteus* isolates isolates from patients in Mansoura University Hospitals, Egypt.

Antimicrobial categories	**Antimicrobial Agents (Antibiotics)**	**Symbol**	**Resistance (R)**		**Intermediate (I)**		**Susceptible (S)**	
			No.	**%**	**No.**	**%**	**No.**	%
Aminoglycosides	Amikacin	AK	30	60	5	10	20	40
Penicillins	Amoxicillin	AMX-AX	48	96	0	0	6	12
Cephalosporins	Ceftazidime	CAZ	49	98	0	0	5	10
	Ceftriaxone	CRO	28	56	10	20	23	46
	Cefurexime	CXM	39	78	0	0	15	30
Fluoroquinolones	Ciprofloxacin	CIP	29	58	5	10	18	36
	Norfloxacin	NX-NOR	25	50	15	30	24	48
Penicillins/ß-lactamase inhibitors	Amoxicillin&clavulanic	AMC	28	56	7	14	23	46
Quinolone	Nalidixic acid	NA	42	84	5	10	11	22
Diaminopyrimidines	Trimethoprime	TR-SXT	31	62	7	14	20	40
Carbapenems	Meropenem	MEM	9	18	0	0	45	90

The results in Table (3) and (4) indicated that the highest no. of isolates (40) were multidrug resistant (MDR).

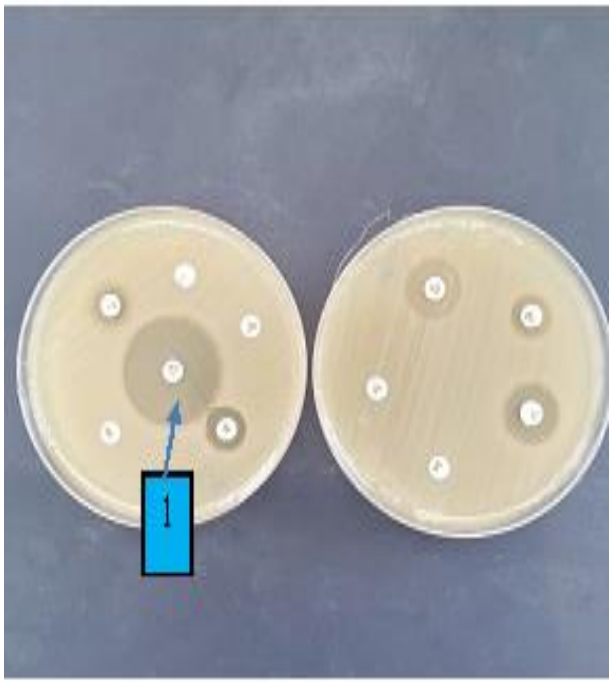

Figure (1) : Multidrug resistant (MDR) *Proteus* spp. Except no.1

Proteus spp. sample is MDR because it exhibits resistance to at least one manager in ≥3 antimicrobial groups, It was resistant to (amikacin from aminoglycoside), (ciprofloxacin from fluoroquinolones), (ceftazidime,cefurexime and ceftriaxone from cephalosporins), (nalidixic acid from quinolone), (amoxicillin from penicillins), (trimethoprime from diaminopyrimidines) and (amoxicillin&clavulanic from Penicillins/ß-lactamase).

The diameter of the inhibition zones of ethanolic extracts were tabulated in Table (5) and shown in Photo (2). Of all cuttings, the ethanolic extract of clove was the most active one with inhibition zones diameter ranged between (14mm – 25 mm), followed by green tea with inhibition zones diameter ranged between (12mm – 21mm). Followed by cinnamon, ginger and fennel respectively

Table (3): Partition of antibiotic resistant *Proteus* spp. isolates into Multi drug resistant (MDR).

***Proteus* spp. isolate No.**	**Resistance**	***Proteus* spp. isolate No.**	**Resistance**
	MDR		**MDR**
1	√	**26**	√
2	√	**27**	√
3	-	**28**	-
4	-	**29**	√
5	√	**30**	√
6	√	**31**	-
7	√	**32**	√
8	√	**33**	√
9	√	**34**	-
10	√	**35**	√
11	√	**36**	-
12	√	**37**	√
13	√	**38**	√
14	√	**39**	√
15	√	**40**	√
16	√	**41**	-
17	-	**42**	√
18	√	**43**	√
19	√	**44**	√
20	√	**45**	√
21	√	**46**	-
22	√	**47**	√
23	-	**48**	√
24	√	**49**	√
25	√	**50**	√

Table (4): Percentage of multidrug resistant *Proteus* spp. isolates (n=50**)**

Multidrug resistant (MDR)	
No. of isolates	**Percentage(%)**
40	80

Table (5): Antibacterial activity of ethanolic plant extracts against the most resistant *Proteus* spp. Isolates.

Resistant *Proteus* spp. isolate No.	**Diameter of inhibition zone (mm) of different ethanolic plant extracts**						
	Fennel	**Green tea**	**Anise**	**Cinnamon**	**Fenugreek**	**Ginger**	**Clove**
1	**0**	**12**	0	15	0	0	20
2	**0**	**17**	0	10	0	0	14
5	**0**	**19**	0	12	0	0	23
9	**8**	**11**	0	18	0	9	21
15	**0**	**13**	0	13	0	0	20
18	**0**	**17**	0	10	0	0	18
21	**0**	**15**	0	15	0	14	20
22	**0**	**12**	0	13	0	0	25
32	**0**	**21**	0	17	0	11	19
35	**0**	**14**	0	0	0	0	16
39	**0**	**17**	0	12	0	0	14
40	**0**	**20**	0	18	0	0	22
44	**0**	**15**	0	15	0	0	14
47	**4**	**12**	0	0	0	0	17
50	**0**	**18**	0	13	0	0	24

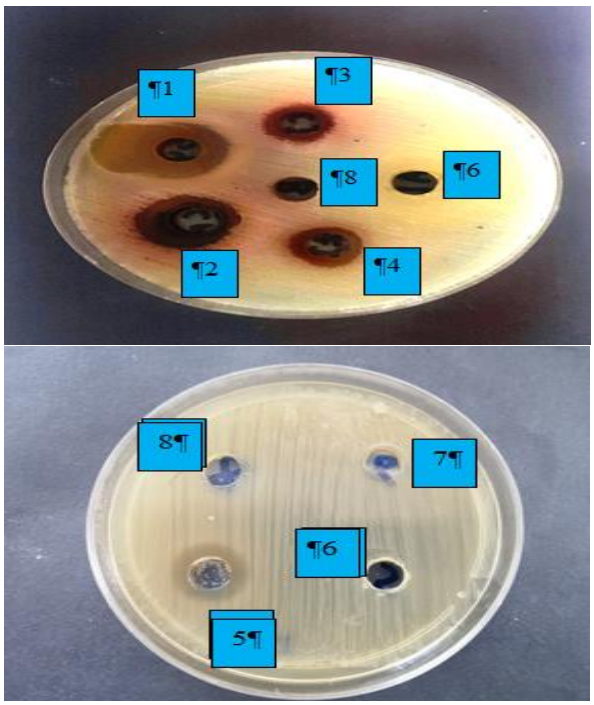

Photo (2): Inhibition zone of different plant extracts against one of the most resistant *Proteus* spp. Isolates

1= Syzygium aromaticum, 2= Cinnamomum verum, 3= Zingiber officinale, 4= Foeniculum vulgare, 5= Camellia sinensis, 6= Pimpinella anisum, 7=Trigonella foenum-graecum, 8= Blank (Dimethylsulfoxide (DMSO).

Discussion

In this exercise, the antimicrobial exposure of 50 *Proteus* spp. detaches against 11 agents from 8 antimicrobial categories. The highest resistance was shown to ceftazidime (98%) and the highest susceptibility was shown to meropenem (90%).

This study also found differences in the rates of susceptibility of *Proteus* spp. isolates to several agents in Fluoroquinolones and penicillins classes. Susceptibility to Ciprofloxacin (36%) was low compared with Norfloxacin (48%) while the susceptibility of Amoxicillin & clavulanic (46%) was in the middle between Ciprofloxacin and Norfloxacin.

In this study, 40 isolates (80%) was documented as MDR among 50 clinical isolates of *Proteus* spp. isolated from patients in Mansoura University Hospitals, Egypt. With a good definition of MDR, there is little published research on multidrug resistance in clinical isolates of *Proteus* spp. in Egypt, and the investigation using our criteria did not find any results. Antibiotics resistance showed in 48% *P. mirabilis* strains confuses the conduct of taints.

The resistant strains are rising sharply and current therapies are becoming unable to manage with the condition [7].

Inside healthcare facilities, the occurrence of amoxicillin-resistant P. mirabilis is close to that of *E. coli* (38% to 48.5%) [14]. *P. mirabilis* and *P. vulgaris* are obviously resistant to polymyxins (colistin), nitrofurans, tigecycline and tetracycline [15]. Medicinal plants tragedy an vital role in the treatment of human infections due to their high antibacterial activity.

Medicinal plants are used specifically in developing countries due to their local availability, high efficiency and their low price [16-17].

Plants are rich in phytochemicals including tannins, alkaloids, terpenoids, and flavonoids which have antimicrobial properties (18-19). In this study, some tested medicinal plant extracts showed good activity against *Proteus* spp. isolates whilst antibiotic therapy had limited effect. The most effective medicinal plant extract against antibiotic resistant *Proteus* spp. isolate was clove.

Conclusion

Fifty *Proteus* spp. isolates were collected from different patient samples admitted to Mansoura University Hospitals. All isolates were identified as *Proteus* spp. by using morphological and biochemical methods. Based on the effect of different antibiotics (11 antibiotics) on the bacterial activity, the result obtained here concluded that the highest resistance was shown to ceftazidime (98%) and the highest susceptibility was shown to Meropenem (90%). Seven ethanolic medicinal plant extracts were separated for their antibacterial activity beside the most resistant *Proteus* spp. isolates by the agar well diffusion method. The ethanolic extract of the *Syzygium aromaticum* was the most active against the most resistant *Proteus* spp.isolates.

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