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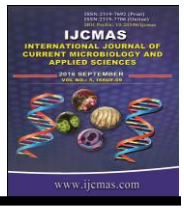

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Multidrug Resistant *Proteus mirabilis* Isolated from Urinary Tract Infection from Different Hospitals in Baghdad City

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A B S T R A C T

Clinical one hundred thirty two samples were collected during the time from 13/9/2015 to 12/11/2015 of urine samples from patients suffering from urinary tract infection (UTI), from different hospitals in Baghdad city. The results of cultural, microscopic, biochemical tests, which was confirmed by API 20E indicated that in urine samples *Escherichia coli* have the highest occurrence frequency followed by *Proteus mirabilis* and *Klebsiella* species. Susceptibility test against twelve antimicrobial agents was done for all of the *Proteus mirabilis* pathogenic isolates (53 isolates). The results displayed that most of the isolates were resistant to Methicillin, methoprim and Rifampin followed by trimethoprim–sulfamethoxazole, chloramphenicol, and cefazoline, while the most effective antimicrobial agents against *P. mirabilis* were Imipenem, Amikacin, Azetronam, Azithromycin, Ciprofloxacin, whereas a moderate affect appeared against both gentamycin and tobramycin.

Introduction

The urinary tract infection (UTI) is defined as infection or colonization of the urinary tract (urethra, bladder, ureter and kidney) by microorganisms. The most common bacterial uropathogens in UTI are*: Escherichia coli, Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis* and *Enterobacter cloacae* (Foxman, 2014).

A Urinary tract infection can occur anywhere along the Urinary tract. It includes urethritis, cystitis, pyelonephritis, epidydimitis, prostatitis, perinephritis, and abscess and considered one among the most common infectious diseases is widely seen among all age groups of individuals. However some groups of people are more prone to UTI than others. For example there is a higher risk to develop UTI in diabetic patients (Chen *et al*., 2009). Females have fourteen times more chance to develop UTI than men.The differences are attributed to involvement of several factors. Such as they has a shorter urethra compare to men which open nearer to the anus so that the lower third of urethra is continually contaminated

with pathogens from vagina and rectum. Women tend not to empty their bladder as completely as men (Minardi, 2011).

Proteus mirabilis is a Gram-negative bacterium which is well-known for its ability to robustly swarm across surfaces in a striking bulls'-eye pattern. Clinically, this organism is most frequently a pathogen of the urinary tract, particularly in patients undergoing long-term catheterization. *P. mirabilis* uses a diverse set of virulence factors to access and colonize the host urinary tract, including urease and stone formation, fimbriae and other adhesins, iron and zinc acquisition, proteases and toxins such hemolysin and its function of pore formation, biofilm formation, and regulation of pathogenesis (Schaffer *et al.,* 2016). The routine use of antibiotics leads to the prevalence of antibiotic resistance and development of antibiotic resistance genes particularly, within gram negative organisms and this consider as the most serious problems in the field of medicine (Sedláková *et al*., 2014). In *P.mirabilis* the antimicrobial resistance is increasing, such as the resistance to extended-spectrum cephalosporin's due to the production of extended-spectrum β-lactamases (ESBLs), which causes epidemiologic effect of *P. mirabilis* bacteremia (Sohn *et al*., 2011). The aim of the study is to Isolate and diagnose the bacteria *P. mirablis* isolated from patients with urinary tract infections and to identify the gene responsible for the production of virulence factor.

Materials and Methods

A total of 132 samples were collected from urine specimens or catheterized urine samples in sterile containers in different hospitals in Baghdad city (Al Kendy hospital, Al Yarmouk hospital, Medicine city/Baghdad teaching hospital, Central Child hospital and Medicin city/Teaching labratory). During the period from 13/9/2015 to 12/11/2015.The samples were cultured on MacConkey agar and blood agar plates, and then incubated for 24 hours at 37◦C.

Identification of isolates

The isolates were identified depending on the morphological and biochemical tests and compared to the scheme described by Holt *et al*., (1994), then confirmed by the Api 20E test.

Microscopic and Morphological Identification

After the isolation of bacteria on MacConkey and blood agar, their shape, size, texture and colony arrangement were observed. Single colonies were picked up, stained with gram stain. Finally they were examined under microscope to identify their shape and length.

Biochemical test

Oxidase Test (Lanyi,1997); Indole test (Cruickshank *et al*., 1975); Blood Hemolysis Test (Collee *et al*., 1996); DNase and Urease test (Atlas *et al*., 1995).

Antimicrobial susceptibility test

Twelve antimicrobials discs were used to detect the sensitivity of 53 isolates of *Proteus mirabilis* according to Bauer *et al*., (1996) method. Bacterial isolates were cultured on MacConkey broth overnight a37◦C. Muller Hinton agar were prepared and distributed into petri dishes. Isolated colonies were suspended in 5 ml normal saline, mixed by vortex. Their turbidity was compared against standard McFarland solution. Aliquot of 2µl of bacterial

suspension was placed on Muller Hinton plates, spread by cotton swab in three different directions by rotating the plate 60◦ for each direction. The plate placed upside down for few minutes at room temperature. A number of antibiotics were placed on the plates and incubated at 37◦C overnight. Inhibition zones were measured in (mm) with zone inhibition ruler, the result compared to the National Committee for Clinical Laboratory Standards.

Results and Discussion

A total of 132 urine samples were collected from different hospitals in Baghdad city (33 from Medicine city /Central Child hospital,21 samples from the Medicine city/Baghdad teaching hospital, 19 from Al-Kindy,14 from Medicine city/Teaching laboratory and 11 from Al-Yarmouk hospital. The primary characterization of Gram negative bacteria depended on gram stain and their ability to ferment lactose on MacConkey agar, swarming on blood agar, as well as on oxidase and indole tests (Holt *et al*., 1994). The prevalence of bacterial isolates in different specimen showed that there was variation in causative agents. the major bacterium was *E.coli* with high occurrence frequency 59 followed by *Proteus mirabilis* in a percentage of 53, *E.facalis* 10, *P.vulgaris* 6 and *Klebsiella* species 4, this result was almost similar to that of Caliskan, (2005) who indicated that the major bacterium was *E. coli*, followed by *P. mirabilis*, *E. faecalis*, *P.vulgaris* and *K. pneumoniae*. Figure(1).

Isolation and identification of *Proteus mirabilis*

Fifty Three isolates of *Proteus mirabilis* were characterized by cultural, microscopic diagnosis, biochemical testes, API 20E system and Vitek 2 system as a confirmatory techniques. The colony morphology of *Proteus* genus was initially identified according to their swarming phenomenon on blood agar, also they have a distinctive fish odor (Figure.2). While Proteus growth on MacConkey appeared to be pale due to incapability of this genus to ferment lactose sugar (O'hara *et al*., 2000).

Biochemical tests

Several biochemical tests were done for the identification of *Proteus mirabilis*. All the fifty three isolates showed positive results for urease, negative results for oxidase (Table 1), these results agreed with (Kishore, 2012; Kamga *et al*., 2012).

For further confirmation of *Proteus mirabilis*, API 20E test were done for the selected isolates, and the results agreed with the biochemical tests and reveled that these isolates were *Proteus mirabilis* table (2).

Prevalence of *Proteus mirabilis* in the Urine samples

The results indicated that *Proteus* species isolates among the total sample isolates were 59, and *Proteus mirabilis* was 53, whereas *Proteus vulgaris* was six (Figure 3). These results confirmed with that reported by Kadhim*et al.*,(2014) how mentioned that among the *Proteus spp*. isolates the percentage of *Proteus mirabilis* 12(85.7%) was more than *Proteus vulgaris* two (14.3%) from total Proteus isolates, this variation may be due to the differences in the size of sample, number of hospitals surveyed, and medication taken before sampling. Also Ismail, (2004) found that the isolation of 67 isolates of *Proteus spp.* from 598 samples of U.T.I. patients from different regions in Yemen and the diagnostic results indicated that 62 isolates (% 92.54) were *Proteus mirabilis* while the remaining 5 isolates (%

7.46) were *Proteus vulgaris*. Since *Proteus mirabilis* have many virulence factors that are vital for inflicting UTI, several of these factors appear to be more important for establishing infection in different areas of the urinary tract (Al-Bassam, 2010). Therefore *Proteus* species isolates percentage from the total number of urine isolates was 59, which was higher in comparable with other samples, and *P. mirabilis* was 53 table (1), figure (1), which was confirmed with that result of Wang *et al*., (2014), who found *P. mirabilis* isolates were (49.4%) from the most isolates were collected from urine, as well as Singh *et al*., (2015) reported that 45 isolates belong to the *Proteus* species was collected from 100 sample of urine. On the other hand a recent study in Al-Diwanyia city produced by Ali and Yousif (2015) found that the *Proteus mirabilis* percentage was (17.64%) in patient with urinary tract infection.

This result shows elevation in the infection by *proteus* spp. among UTI patients, because this species especially *Proteus mirabilis* is normally found in the human intestine along with other organisms composing a highly complex micro flora, they also inhabit other outside environments, and are especially prevalent in hospitals and care facilities, the skin and mucus of both patients and personnel working in these environments, which may be the primary vectors for pathogenicity, plus this bacteria metabolically involved in urease production which act as one of the reasons the pathogen is successful in colonizing the urinary tract and cause infection in humans (Kearns, 2010), furthermore motility of this isolate is highly complex, which is called swarming, is a primary factor in the success of *Proteus mirabilis* in causing complicated UTI and other more serious bladder and kidney infections.

In addition to their adaptive mobile abilities, other virulence factors have deemed *Proteus mirabilis* successful UTI causative agents. Overtime their inhabitance in hospitals has led to the expression of several antimicrobial resistance genes making infections very difficult to treat (Carey *et al*., 2013).

Antimicrobial susceptibility of *P.mirabilis*

Depending on NCCLs (2007) guideline, Fifty Three isolates were tested against twelve antimicrobials discs. Differences in the antimicrobials resistance were observed. Most of the isolates were resistant to Methicillin (95.6%), and Rifampin (91.8%). while the most effective drugs against *P. mirabilis* were Imipenem, Amikacin, Azetronam, Azithromycin and Ciprofloxacin.

Imipenem was the most effective drug against *P.mirabilis*isolates (97.2%) was sensitive, Figure (4). This result was agreed with Shaaban *et al*., (2012), as well Yassen and Khelkal, (2015) were documented that the sensitivity of *P.mirabilis* is 100% to this antimicrobial. Some *Proteus mirabilis* isolates present an elevation in the resistance level to imipenem due to many reasons: the loss of outer membrane porins, decreased expression of PBP1a or reduced binding of imipenem by PBP2 (Girlich *et al*., 2014). Tsai *et al*., (2015) noted that the development of resistant against imipenem in *Proteus mirabilis* is due to the absence of 24 kDa OMP.

Amikacin is an amino-glycoside antibiotic used to treat different types of bacterial infection. It is works by binding to the bacterial 30s ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth. In this study *P.mirabilis* isolate show high sensitivity to amikacin (92.6%), Figure (4). These result confirmed with result by Singla

et al., 2015 found the percentage of susceptibility to amikacin was 87% to 98%, which considered an agreement to this result.

In this study *P. mirabili* isolates showed high sensitivity against azetronam (81.7%) Figure (4), these results were confirmed to that reported by Al-Bassam, 2010 who detected (81%) sensitivity to this antimicrobial. Stock (2003) showed that *P.mirabilis* are naturally sensitive to azetronam, Whereas Yassen and Khelkal, (2015) reported an intermediate sensitivity.

In the presented study (72.7%) of *Proteus mirabilis* isolates were sensitive to azithromycin see Figure (4), which agrees with a study in India by Manikandan and Amsath, (2013) showed 75% sensitivity to this antimicrobial in *Proteus* species isolates.

Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluroquinolone drug class(Nelson *et al*, 2007), it is member of the broad spectrum antimicrobial agents, which inhibits bacterial DNA and protein synthesis and considered an important drug in the treatment of urinary tract infection. In this study (67.4%) of the isolates were sensitive to Ciprofloxacin (Figure 4). A Study in Taiwan by Wang *et al*., (2014) reported that the susceptibility to this antimicrobial decreased (from 80.1% to 53.8%), which is considered approximately in an agreement to the result of this study, about the development of resistant.

Gentamycin had a moderate activity on *P.mirabilis* isolates (55.8%) see Figure (4), cause it belong to Aminoglycosides that are powerful broad spectrum antimicrobials, which are inhibitors of protein synthesis in prokaryote. A study in Taiwan by Wang *et al*. (2014) demonstrated that the rate of susceptibility to gentamicin was decreased to (57.7%), which agrees with this study.

Tobramycin result showed (56.5%) of the isolates were sensitive to this antimicrobial, Figure (4). A study by Alshwaikh *et al*., (2014) found the percentage of resistance was (40%), which considered an agreement to this result, Whereas Patil. (2014) reported that the tobramycin was not able to inhibit the growth of *proteus* species more than 34%.

Table.1 Biochemical test for *P.mirabilis* and *P.vulgaris* isolates from urine specimens collected from patients with acute UTI

Biochemical test	*P.mirabilis*	*P.vulgaris*
Oxidase	Negative	Negative
Catalase	Positive	Positive
Indole	Negative	Positive
Vogas-Proskauer	Negative	Negative
Urease	Positive	Positive
Citrate Utilization	Positive	Positive
Triple-Sugar Iron Agar	K/A++	K/A++
Methyl Red	Positive	Positive

Table.2 Results of Api20 E diagnostic test for *Proteus mirabilis*

Tests	Substrate	*Proteus mirabilis*
ONPG	Ortho-nitrophenylGaactoside	-
ADH	Arginine dehydrolase	-
LDC	Lysine decarboxylase	-
ODC	Ornithine decarboxylase	+
CIT	Citrate	+
H2S	Sodium Thiosulfate	+
URE	Urea	+
TDA	Tryptophan deaminase	+
IND	Indol	-
VP	Voges-proskauer	-
GEL	Kohn's Gelatin	+
GLU	Glucose	+
MAN	Mannitol	-
INO	Inositol	-
SOR	Sorbitol	-
RHA	Rhamnose	-
SAC	Sucrose	-
MEL	Melibiose	-
AMY	Amygdalin	-
ARA	Arabinose	-

Positive result(+) (-) Negative result

Fig.1 Number of bacterial isolates from urine sample.

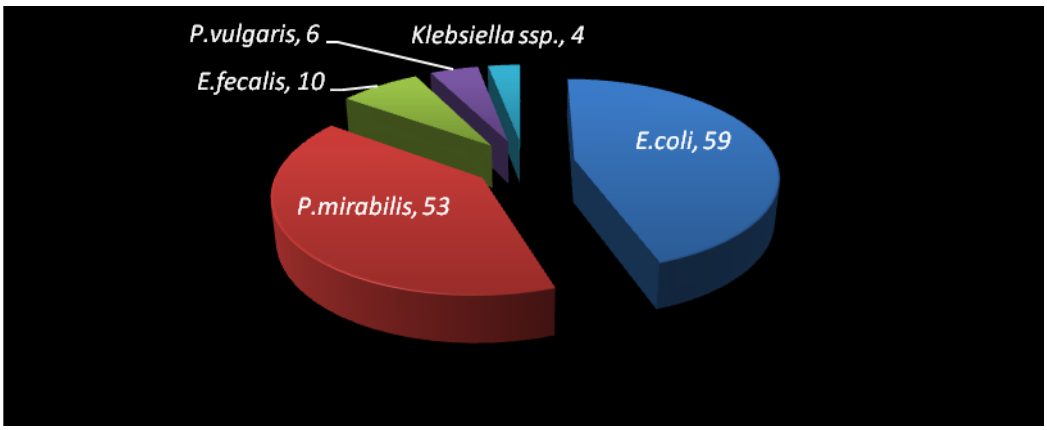


Fig.2 Proteus mirabilis on Blood agar

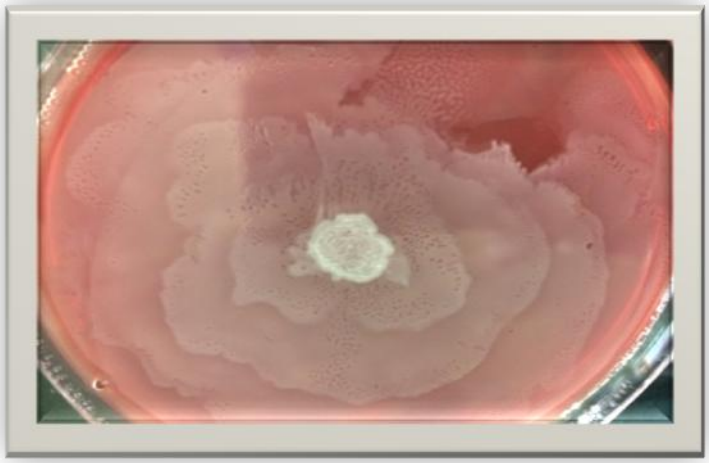


Fig.3 The percentage of *P.mirabilis* isolate and *P.vulgaris* isolated from patient suffering from UTI.

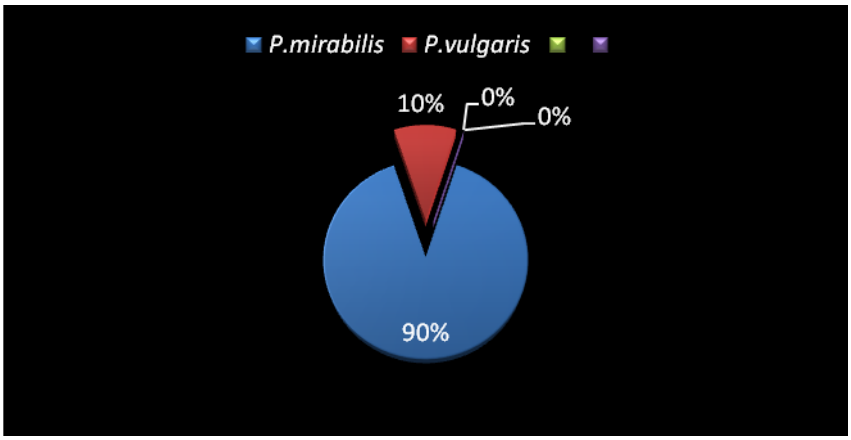
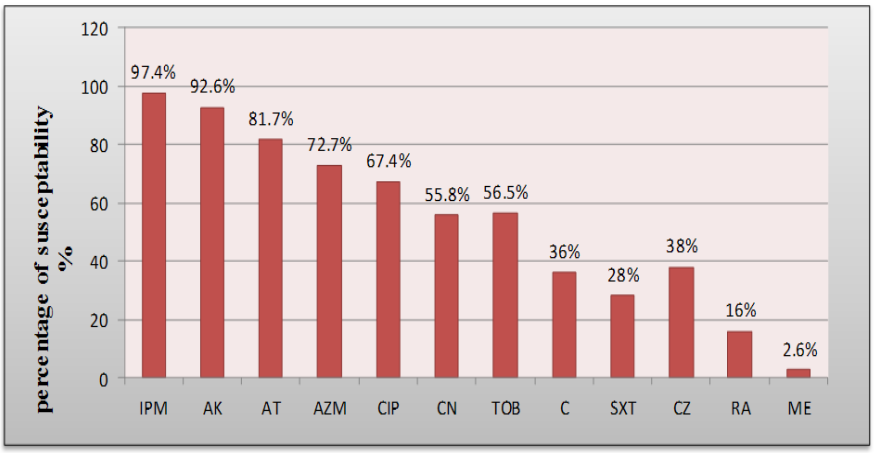


Fig.4 The antibiotic susceptibility test of *P.mirabilis* isolates.



P.mirabilis showed (36%) sensitivity to chloramphenicol in this study see (Figure 4), chloramphenicol is a bacteriostatic antimicrobial. It is a most common broad-spectrum antibiotic, alongside the tetracycline. it is effective against a variety of Gram-negative and Gram-positive bacteria, including most anaerobic organisms. It prevents the peptidyl bond formation between the amino acids of growing polypeptide chain. Mordi and Momoh, (2009) demonstrated 28% sensitivity of *P. mirabilis* that agree with this study.

Trimethoprim and sulfamethoxazole are combined together due to their synergism effect on bacteria. It's abroad spectrum bactericidal antimicrobial agent for both gram positive and gram negative bacteria. Trimethoprim is a diaminopyrimidine, whereas sulfamethoxazole is a sulfonamide and the Co-trimoxazole inhibits the synthesis of tetrahydrofolic acid, which is necessary for the synthesis of bacterial nucleic acid along with two components of the drug inhibiting different steps in the folate synthesis pathway (Ramlakhan *et al*., 2014). In this study *P. mirabilis* isolates developed high resistance (72%) against trimethoprim–sulfamethoxazole see Figure (4), which was approximately confirmed with Nahar *et al*., (2014) reported a resistant percentage of (66.7%).

Cefazolin, which is member of cephalosporin it is one of a drug that used for the treatment of *P. mirabilis* infection, but this study showed the pathogenic isolates appeared (62%) resistance to cefazolin see Figure (4). Alkalifawi, (2015) documented that resistant *P. mirabilis* isolates about (84%), this differentiation between both local studies may be due to the number of experimental urine samples, the type and site which were samples was taken, *P.mirabilis* show high resistant against Rifampicin 94% (Figure 4), Raghada*et al*, (2013) reported that *P. mirabilis* isolates had moderate resistant against it, which disagreed with this result, whereas Al-Jawadi (2007) found that *P. mirabilis* isolates emerged 100% resistant to the same antimicrobial agent. Resistance of *P.mirabilis* against methicillin in a percentage 97.4% see Figure (4), this resistance may be due to increasing in the prevalence of plasmid-mediated extended-spectrum β-lactamases (ESBLs) in members of the family Enterobacteriaceae which has become a serious clinical problem on a worldwide scale(Nagano *et al*., 2003), on other hand them over use of this antimicrobial agent.

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